

Gunvor Ekman-Ordeberg

RIPENING OF CERVIX UTERI IN PREGNANCY.

CLINICAL AND EXPERIMENTAL STUDIES
WITH SPECIAL REFERENCE TO TERM
LABOR INDUCTION WITH PROSTA-
GLANDIN E₂ IN VISCOUS GEL

Malmö 1982

Lund

RIPENING OF CERVIX UTERI IN PREGNANCY.

CLINICAL AND EXPERIMENTAL STUDIES

WITH SPECIAL REFERENCE TO TERM

LABOR INDUCTION WITH PROSTA-

GLANDIN E₂ IN VISCOUS GEL

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds
Universitet för avläggande av medicine doktorsexamen kommer att
offentligen försvaras i Kvinnoklinikens föreläsningssal, Allmänna
Sjukhuset i Malmö, fredagen den 10 december 1982 kl. 09.15

av

Gunvor Ekman-Ordeberg

med lic, Øg

Organization LUND UNIVERSITY		Document name DOCTORAL DISSERTATION	
Department of Obstetrics and Gynecology, University Hospital, S-214 01 Malmö, Sweden		Date of issue 1982 12 10	
Author(s) Gunvor Ekman-Ordeberg		CODEN: LUMEDW/(MEKM-1007)/1-49 (1982)	
		Sponsoring organization	
Title and subtitle Ripening of cervix uteri in pregnancy. Clinical and experimental studies with special reference to term labor induction with prostaglandin E ₂ in viscous gel.			
Abstract Spontaneous cervical ripening at term is recognized as softening, effacement and dilatation. Failure in this ripening process is a bad prognostic sign indicating prolonged labor and post term delivery. - The aim of the present investigation was to study cervical priming after local application of PGE ₂ in a new viscous gel and to investigate biochemical changes in cervical connective tissue during spontaneous and PGE ₂ -induced cervical ripening. - After intracervical (i.c.) application of 0.5 mg PGE ₂ in a new gel based on a crosslinked starch powder, favorable cervical states were achieved in 70% of term pregnant women with unripe cervix 5 hours after application of the gel. Intravenous infusion with oxytocin could then be administered without myometrial hyperstimulation. The outcome of the gel treatment was mainly related to the original cervical state and less to gestational duration and parity. Comparing i.c. application of 0.5 mg PGE ₂ with intravaginal (i.vag.) of 4 mg PGE ₂ in gel, the i.c. route was superior to the i.vag. in women with highly unfavorable cervix whereas the treatments were equipotent in women with moderately unripe cervix. The myometrial activity, registered extraamniotically with microtransducer technique, was significantly increased after i.vag. but not after i.c. application. No side effects were found after i.c. application, but 3 women reported episodes of vomiting after i.vag. treatment. - Connective tissue in cervical biopsies taken from the lower part of the uterine cervix from non-pregnant and pregnant women at various gestational age were analysed for collagenolytic activity (DNP-peptide hydrolytic activity and leucocyte elastase), concentrations of collagen (hydroxyproline), glycosaminoglycans, hyaluronic acid, and water. During pregnancy and parturition, an increase in the collagenolytic activity and a congruent decrease in the concentration of hydroxyproline (collagen) was found. The concentrations of glycosaminoglycans and hyaluronic acid were significantly lowered at term. After PGE ₂ -gel treatment, the collagenolytic activity in cervical connective tissue was significantly increased compared to controls.			
Key words Cervix uteri/drug effects; labor, induced; cervix uteri/connective tissue; collagenase; leucocyte elastase; collagen; glycosaminoglycans; hyaluronic acid, Prostaglandin E/administration and dosage; gels; oxytocin.			
Classification system and/or index terms (if any)			
Supplementary bibliographical information			Language English
ISSN and key title			ISBN
Recipient's notes		Number of pages 49	Price
		Security classification	

Distribution by (name and address) **Gunvor Ekman-Ordeberg, Department of Obstetrics and Gynecology, University Hospital, S-214 01 Malmö, Sweden.**

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Date 82 10 22

From the Department of Obstetrics and Gynaecology,
University of Lund, Malmö General Hospital,
S-214 01 Malmö, Sweden.

RIPENING OF CERVIX UTERI IN PREGNANCY.

CLINICAL AND EXPERIMENTAL STUDIES

WITH SPECIAL REFERENCE TO TERM

LABOR INDUCTION WITH PROSTA-

GLANDIN E₂ IN VISCOUS GEL

by

Gunvor Ekman-Ordeberg

Malmö 1982



*Trust in the Lord with all your heart,
on your own intelligence rely not;*

Proverbs 3:5

*The beginning of wisdom is the fear of
the Lord, and knowledge of the Holy One
is understanding.*

Proverbs 9:10

*To my dear Gunnar, Rakel,
Tomas and Jakob.*

CONTENTS

List of publications	7
Acknowledgements	8
Introduction	9
Aim of the study	17
Material and methods	18
Patients	18
Gel preparation	18
Biochemical analyses	22
Results	25
Discussion	34
Conclusions	41
References	43
Publications:	
I Intracervical application of prostaglandin gel for induction of term labor.	50
II The impact on labor induction of intracervically applied PGE ₂ -gel, related to gestational age in patients with an unripe cervix.	65
III Intracervical application of PGE ₂ -gel combined with early intravenous infusion of oxytocin for induction of	

term labor in women with unripe cervix.

75

IV Intravaginal versus intracervical application of prostaglandin E_2 in viscous gel for cervical priming and term labor induction in patients with an unfavorable cervical state.

92

V Ripening of the human uterine cervix related to changes in collagen glycosaminoglycans and collagenolytic activity.

113

VI Increased collagenolytic activity post partum in cervical connective tissue from women treated with prostaglandin E_2 .

131

PUBLICATIONS

The present thesis is based on the following publications:

- I Ulmsten, U., Wingerup, L., Belfrage, P., Ekman, G., and Wiqvist, N.: Intracervical application of prostaglandin gel for induction of term labor. *Obstet Gynecol* 59:336, 1982.
- II Ekman, G., Persson, P.H., Ulmsten, U., and Wingerup, L.: The impact on labor induction of intracervically applied PGE₂-gel, related to gestational age in patients with an unripe cervix. Accepted for publication in *Acta Obstet Gynecol Scand*. In press.
- III Ekman, G., Ulmsten, U., and Wingerup, L.: Intracervical application of PGE₂-gel combined with early intravenous infusion of oxytocin for induction of term labor in women with unripe cervix. Accepted for publication in *Arch Gynaekol*.
- IV Ekman, G., Forman, A., Maršál, K., and Ulmsten, U.: Intravaginal versus intracervical application of prostaglandin E₂ in viscous gel for cervical priming and term labor induction in patients with an unfavorable cervical state. Submitted for publication.
- V Uldbjerg, N., Ekman, G., Malmström, A., Olsson, K., and Ulmsten, U.: Ripening of the human uterine cervix related to changes in collagen, glycosaminoglycans and collagenolytic activity. Submitted for publication.
- VI Ekman, G., Uldbjerg, N., Malmström, A., and Ulmsten, U.: Increased collagenolytic activity post partum in cervical connective tissue from women treated with prostaglandin E₂. Accepted for publication in *Gynecol Obstet Invest*.

In the text, these papers are referred to by their Roman numerals.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to:

Professor Stig Kullander, Head of the Department of Obstetrics and Gynecology, Malmö General Hospital, University of Lund, for his personal interest and support during the study.

Professor Ulf Ulmsten, my friend and teacher, for never failing generous support and help throughout the investigations.

Professor Sven Gardell, Head of the Department of Physiological Chemistry, University of Lund, for his personal interest and support during the study and for his generosity with laboratory facilities.

My co-authors for excellent collaboration.

All colleagues, midwives, nurses and other members of the staff of the Department of Obstetrics and Gynecology for their enthusiastic help throughout the investigations.

Ms. Ann-Christin Flyving and Ms. Ulla Andersson for skillful preparation of the manuscript and excellent type-writing.

Ms. Ulla-Britt Stenberg for all-round, kind support and assistance.

The investigations were supported by grants from the Medical Faculty, University of Lund, Sweden, Danish Medical Research Council (J.Nos.12-2264), and from the Foundation of Allmänna BB, Stockholm.

INTRODUCTION

Assessment of the functional state of cervix uteri has for a long time been recognized as most important when evaluating the progress of human pregnancy and parturition. Several investigations have shown that cervical ripening by means of effacement and dilatation indicates the approaching spontaneous labor (Bishop 1964, Anderson & Turnbull 1969, Hendricks et al. 1970).

Abnormalities in the ripening process may compromise both mother and child. Thus, an extensive cervical ripening before term may lead to abortion or preterm delivery (Bengtsson et al. 1968), whereas insufficient ripening at term is a bad prognostic sign indicating prolonged labor or postterm delivery (Calder 1979, Vorherr 1975).

Although considerable attention has been paid to the cervical state in clinical routine, most scientific research concerning spontaneous and induced labor has been directed to the contractile state of the myometrium. In recent years there has been an increased interest in methods improving an unfavorable cervical state thereby facilitating subsequent labor induction. To some extent, this may be due to an increased frequency of term labor inductions but also to the clinical experience that, in patients with unfavorable cervixes, conventional methods for labor induction, like intravenous infusion of oxytocin

and/or amniotomy, are ineffective and afflicted with high frequency of fetal distress and instrumental deliveries. (Garrett 1960, Turnbull and Anderson 1968, Calder 1979, Shepherd et al. 1981).

Despite a growing scientific interest, our knowledge of the priming process of the human cervix is still very incomplete both from morphological and biochemical aspects. Moreover, little information exists on the functional interplay between the myometrium and the cervix during both spontaneous and pharmacologically induced cervical ripening.

Due to an extensive work by Danforth, the pioneer in this field, we know that the human cervix is composed mainly of fibrous connective tissue (Danforth 1947). The smooth muscle which surrounds the connective tissue constitutes 10 - 15 % of the total amount of the organ (Danforth 1954, Schwalm and Dubrausky 1966,). Furthermore, Rorie et al. have found that the distal part of human cervix uteri contains less (6 %) muscular tissue than the proximal part of the organ (29 %) (Rorie et al. 1967).

Considering the biochemical aspects, the basic molecule of cervical connective tissue is collagen of which 70 % is of type I and 30 % of type III (Ito et al. 1979a). The collagen fibrils are held together by crosslinks. Newly synthesized collagen without crosslinks is extractible from

tissue samples with neutral salt solutions. Some cross-links are unstable at low pH. Collagen with such cross-links can therefore be extracted with acetic acid (van der Rest 1980). Digestion of the tissue with pepsin is often necessary to enhance the extractability (Ito et al. 1979a).

Collagen is resistant to most proteinases except to collagenase and leucocyte elastase. The products after cleavage of collagen are denaturated at 37°C and then further degraded by other proteinases (Woolley and Evanson 1981).

Leucocyte elastase which is a serine proteinase, localized to the azurophilic granulae of polymorphonuclear granulocytes, can also degrade collagen (Ohlsson 1980).

Elastin is also found in human cervical connective tissue, but very little is known about its functional role for the ripening process (Leppert et al. 1982).

Important molecules in all connective tissue are proteoglycans. They are made up by a number of glycosaminoglycans (acid mucopolysaccharides) connected to a protein core. A glycosaminoglycan is a long chain (20 - 40 kDa) of repeating disaccharides containing one hexosamine and one uronic acid. (Lindahl and Höök 1978, Caplan and Hascall 1980).

The dominating proteoglycan in cervical connective tissue contains dermatan sulphate. Fifteen per cent is hyaluronic acid and the remaining 15 % is heparan sulphate proteo-

glycan. The latter may originate from the vessels (Uldbjerg et al. 1982a, Uldbjerg et al. 1982b, Kitamura et al. 1980b).

An interaction between collagen and proteoglycans has been shown by physicochemical measurements (Öbrink 1973) and by electron microscope examinations (Scott and Orford 1981).

Existing information about biochemical reactions in human cervical connective tissue during spontaneous ripening and parturition is sparse and inconsistent. Furthermore, in most of the investigations the post partum cervical connective tissue is solely compared with the nonpregnant state (Nakaya 1973, Danforth et al. 1974, Karube et al. 1975).

Since several investigators have found that oxytocin alone or in combination with amniotomy is inefficient for labor induction in patients with an unripe cervix, other agents have been looked for (Garett 1960, Turnbull and Anderson 1968). Much interest has been focused on the prostaglandins. Partly this is due to their possible role in onset of spontaneous labor, (Gustavii 1975, Liggins et al. 1977, Mitchell 1981) but also to their well-documented efficacy in medical termination of early pregnancy (Bygdeman 1978, Bygdeman 1980).

However, when prostaglandins are to be used as pharmacological agents in obstetrics and gynecology, several problems will arise related to, e.g., the pharmaceutical

preparation of the drugs and to the way of administration. Systemic administration is associated with considerable maternal side effects especially from the gastrointestinal tract (Karim and Sharma 1971, Embrey 1975, Karim et al. 1980). Local administration of prostaglandins on the other hand has been reported effective for cervical priming with a low frequency of maternal side effects. Compared to prostaglandin $F_{2\alpha}$, prostaglandin E_2 (PGE_2) has been considered more favorable both for cervical priming and for induction of term labor (Najak et al. 1970, Embrey 1970, MacKenzie and Embrey 1979).

Thus several investigations have reported a high frequency of cervical priming after extraamniotic or intracervical application of a single low (0.25 - 0.5 mg) dose of PGE_2 in viscous gel (for review see Ulmsten and Wingerup 1979).

Despite high efficacy, the conventional cellulose gel preparations for local administration of prostaglandins are not perfect from a pharmaceutical point of view. Thus, several factors such as instability of the prostaglandin compound, inhomogeneity, and risk for bacterial contamination can compromise the preparation (Karim et al. 1968, Brummer et al. 1971, Roseman et al. 1973, Srivastava et al. 1973). Due to this, there is a need for an improved PGE_2 -gel preparation which could be industrially and aseptically produced.

Promising results concerning both cervical priming and term labor induction have been reported after intracervi-

cal application of 0.5 mg PGE_2 in a new gel based on a crosslinked starched polymer (Ulmsten et al. 1979, Ulmsten and Wingerup 1980). However, there is still an urgent need for further controlled studies with this new PGE_2 gel preparation.

Also other factors than the quality of the gel preparation may be of importance for the effect of PGE_2 gel treatment. It is wellknown that the spontaneous cervical ripening is correlated to the gestational duration (Hendricks et al. 1970). So far, no investigation has dealt with the importance of gestational length for the outcome of PGE_2 -gel treatment in patients with unripe cervix. This is probably due to lack of methods for proper estimation of gestational age. However, the development of the ultrasound technique enables a more accurate estimation of the duration of gestation (Campbell et al. 1971, Persson et al. 1978). Hereby, it has become possible to elucidate the impact of gestational duration on PGE_2 -induced cervical priming in term pregnant women with unripe cervix.

Probably for practical reasons, assessment of cervical state after local PGE_2 -gel treatment has previously not been performed until 16 - 24 hours after gel application (Shepherd et al. 1976, Calder et al. 1977, Thiery 1977, Ulmsten and Wingerup 1979). It is, however, of great clinical interest to estimate more exactly how soon a

favorable cervical state can be achieved after PGE₂-gel application.

Moreover, subsequent induction or augmentation of labor with intravenous infusion of oxytocin has hitherto not been performed until 16 - 24 hours after administration of PGE₂ gel. This rather long delay is probably due to the reported possible risks for uterine hypercontractility (Gillespie 1972, Gillespie et al. 1972, Thiery 1979).

High efficacy and few side effects have been reported after extraamniotical and intracervical PGE₂ gel application for cervical priming, (Calder et al. 1977, Ulmsten and Wingerup 1979) but these routes may be considered more complicated than the simple intravaginal application. However, for intracervical treatment only a small amount of PGE₂ (0.5 mg) is necessary, whereas the intravaginal application requires a considerably higher dose (2 - 5 mg) to obtain similar clinical effects (MacKenzie and Embrey 1977, Gordon-Wright and Elder 1979a). Due to this and to the prompt absorption of the applied PGE₂-compound from vagina into systemic circulation, the intravaginal route of application should be afflicted with more side effects, such as gastrointestinal discomfort and uterine hypercontractility (Gordon-Wright et al. 1979b, Davey et al. 1980, Karim 1980).

Hitherto, no prospective, comparative studies have been published on intracervical and intravaginal application of PGE₂ in viscous gel for cervical priming and term labor induction.

In several investigations, a prominent cervical ripening is reported after intracervical or intravaginal application of PGE_2 in viscous gels (MacKenzie and Embrey 1977, Gordon-Wright and Elder 1979a, Ulmsten et al. 1979, Ulmsten and Wingerup 1980, Shepherd et al. 1981). However, as in spontaneous ripening, very little is known about the mechanisms causing the ripening process, e.g., if it is associated with local effects within the cervical connective tissue or if it occurs as a result of an increased myometrial activity.

It is obvious from this presentation that several questions remain to be answered concerning both spontaneous and pharmacologically induced cervical ripening in man. The primary aims of the present investigation were as follows:

1. To investigate in a controlled study the cervical ripening effect of intracervical application of 0.5 mg prostaglandin E_2 in a new gel based on a crosslinked starch polymer.
2. To investigate in patients with unfavorable cervix around term the impact of gestational age on the outcome of cervical priming and induction of labor after intracervical application of 0.5 mg prostaglandin E_2 in viscous gel .
3. To investigate the time factor in PGE_2 -induced cervical ripening and to find out whether intravenous infusion of oxytocin could be instituted closely to the PGE_2 gel treatment.
4. To compare the effect of intravaginally and intracervically applied prostaglandin E_2 in viscous gel for priming of the cervix and/or induction of term labor in patients with an unripe cervix, and to quantitate properly the myometrial response of the two different techniques of PGE_2 gel application.
5. To study possible biochemical changes in cervical connective tissue during spontaneous and PGE_2 -induced cervical ripening.

Patients

Before participating in the studies, all women were informed about the purpose and the procedures of the investigations and gave their consent. The PGE₂ gel was applied to term patients with unfavorable cervix (I-IV, VI). All patients had head presentations and intact membranes. The indications for induction were always obstetric, e.g., toxemia, isoimmunization, suspected placental insufficiency, and fetal growth retardation. In the experimental investigations (V,VI), also non-pregnant, menstruating women and early pregnant patients participated.

Gel preparation: (I - IV, VI)

A fresh solution of PGE₂ (Upjohn, USA) in 80 % aqueous ethanol was filtered through a Fluoropore 0.2 u filter. This solution was aseptically added to a sterilized powder consisting of a crosslinked starch polymer (Perstorp AB, Sweden). After homogenization and lyophilization, portions of 0.7 g of the powder containing a predetermined amount of PGE₂ were dispersed and stored at +4°C. In the present studies, 0.5 mg PGE₂ per 0.7 g (I - IV, VI) and 4 mg per 1.0 gram (IV) starch powder was used.

Before application, the PGE_2 containing powder was mixed with physiological saline. To the portions containing 0.5 mg PGE_2 , 2.5 ml saline were added in studies I-II, whereas only 2 ml were used in studies III-IV and VI in order to produce a firmer gel more easy to apply strictly within the cervical canal. For intravaginal application (study IV), 3 ml saline were added to the powder portion containing 4 mg PGE_2 .

Intracervical application (I-IV, VI)

With the patient in semilithotomy position, we applied 0.5 mg PGE_2 in viscous gel intracervically using a 12 cm long semiflexible plastic catheter with an outer diameter of 3.3 mm. During slow withdrawal of the catheter, the gel was instilled in the cervical canal starting just below the internal os. Immediately after application, the catheter was removed.

Intravaginal application (IV)

With the same type of catheter as used in intracervical application, we administered 4 mg PGE_2 in 3 ml gel into the posterior fornix. Immediately after application, the catheter was withdrawn.

In all studies, the patient was staying in bed for 60 minutes after gel application.

Management of patients

In all term patients at least two ultrasound scannings in the 17th and the 32nd week of pregnancy were carried out. Hereby patients with low implanted placenta, breech presentation, and multiple pregnancy were excluded. The ultrasound fetometry in the first half of pregnancy enabled a proper estimation of the gestational age (Campbell 1971, Persson et al. 1978). In all term pregnant women admitted for induction (I-IV, VI), cervical states were assessed in the morning immediately before treatment. We used a modification of Bishops original score. This score has a range from 0 - 10 points, and a score ≤ 5 indicates an unfavorable cervix (Ulmsten and Wingerup 1979).

All term patients were continuously supervised by external cardiotocography (CTG) (Hewlett - Packard) from 30 minutes before until 60 minutes after application of the gel. Labor was considered established when regular uterine contractions were registered with a mean duration of at least 1 minute and with a frequency of 3 per 10 minutes.

Amniotomy was not performed until labor was established and the cervix dilated to ≥ 4 cm. After amniotomy, internal CTG was used. pH measurements on fetal scalp blood were performed whenever indicated.

Myometrial hypercontractility was defined thus: uterine contractions occurring more frequently than 5 per 10 minutes, contraction amplitudes exceeding 60 mm Hg, and resting pressures between contractions exceeding 15-20 mm Hg.

In undelivered patients, reassessment of cervical states was carried out in studies I and II only once, i.e., 24 hours after application of the PGE₂ gel while in studies III, IV, and VI, cervical states was followed continuously with vaginal examinations 5, 24, 29, and 48 hours after the gel application.

In patients not in labor but with favorable cervical states after PGE₂ gel treatment (I-IV, VI), intravenous infusion of oxytocin was instituted using an electronic infusion pump. The infusion started with 2 mU/minute and was subsequently increased by 2 mU/minute every half hour up to 24 mU/minute. This infusion rate was never exceeded.

For proper registration of myometrial activity after intracervical or intravaginal gel-application (IV), intra-uterine pressure (IUP) was recorded by extraamniotically applied microtransducers according to a technique described in detail elsewhere (Ulmsten et al. 1979, Ulmsten and Anderson 1979).

The uterine activity was calculated in Montevideo Units (Krapohl et al. 1970).

Sampling procedures

In non-pregnant women undergoing hysterectomy, cervical biopsies were taken from the distal portion of cervix. Furthermore, cervical biopsies from non-pregnant women undergoing curettage were taken transvaginally from the distal part of cervix. We used a rotating needle with a diameter of 3 mm and with a penetration depth of 12 mm.

In women admitted for therapeutic abortion in first trimester of pregnancy, the cervical biopsies were obtained with the same rotating needle as described above.

In term pregnant women undergoing elective caesarean sections, and in spontaneously delivered women, cervical biopsies were taken vaginally by the help of scissors and tweezers.

All specimens were immersed into liquid nitrogen within 3 minutes after sampling and then stored at -60°C . until biochemical analyses.

Water and glycosaminoglycans

The water content of the biopsies was defined as the difference between the wet weight and the dry weight. The latter was determined after heating the sample at 110°C in vacuum over P_2O_5 for at least five hours, after which a constant weight was obtained. The dry sample was delipidated with acetone and digested with papain. The digest was

applied at a column with 1 ml DEAE cellulose in acetate form. Hyaluronic acid was eluted with 1.1 M pyridine acetate pH 5 and sulphated glycosaminoglycans with 3.0 M pyridine acetate pH 5, and lyophilized. The fraction containing hyaluronic acid was subjected to alkaline degradation according to Carlsson and applied to a column containing 0.5 ml DEAE cellulose (Carlsson 1968). The oligosaccharides from mucin glycopeptides were eluted with 0.4 M pyridine acetate and the purified hyaluronic acid with 1.2 M pyridine acetate. Sulphated glycosaminoglycans and hyaluronic acid were hydrolyzed in 6 M HCl, 100°C for eight hours, lyophilized, and quantitated as hexosamine (Antonopoulos et al. 1964).

Collagen concentration and extractability

The biopsies were cut into pieces smaller than 2 mm and delipidated with acetone. They were initially extracted three times with 1 ml 0.5 M acetic acid and then three times with 1 ml 0.5 M acetic acid containing pepsin (1 mg/ml) (Ito et al. 1979a). Extractions (23 hours) and centrifugations (one hour at 20,000 x g) between these were performed at 4°C. The material was hydrolyzed in 2 ml 6 M HCl at 100°C for 17 hours, lyophilized, and quantitated as hydroxyproline according to Stegeman (Stegeman and Stalder 1967).

Collagenolytic activity

Extractions were performed essentially as described by

Kitamura et al. (1980a). The biopsy was crushed by 10 tons pressure during cooling with liquid nitrogen and then homogenized in 13 volumes of 0.25 % Triton X-100, 50 mM Tris pH 7.3 containing 100 mM CaCl_2 in a TRI-R Tissue homogenizer, S61 for four minutes at 3,000 rpm. The homogenate was centrifuged at 15,000 x g for 20 minutes at 2°C, whereafter the supernatant was diluted with extraction buffer. The hydrolytic activity against DNP-peptide was then assayed as described by Masui et al. (1977). The incubation time was 2 hours and the extraction buffer was used as blank.

Leucocyte elastase was determined in the same extractions with a RIA-method (Ohlsson and Olsson 1978).

Chemical reagents

Papain (twice crystallized, 16 - 40 BAAE units/mg) was purchased from Sigma Chemical Co., U.S.A., DEAE cellulose ion exchanger (DE-52) from Whatman Ltd., England, and 2,4 dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg (DNP-peptide) from the Protein Research Foundation, Japan. All other chemicals were of analytical grade.

Statistical methods

Fisher's exact test (I), Yates' test (IV), and Student's t-test (V, VI) were used for the statistical evaluations.

RESULTS

Intracervical application of prostaglandin E₂ in a new gel for induction of term labor (I)

In a randomized double-blind study, 50 nulliparous term pregnant women were given either 2,5 ml gel containing 0.5 mg PGE₂ (PGE₂-gel) or 2,5 ml gel without PGE₂ (placebo gel). Eleven out of 25 women (44 %) receiving PGE₂ gel were induced into labor and delivered within 24 hours, compared to only 2 women out of 25 (8 %) in the placebo group. This difference is statistically significant ($p < 0.01$).

In the remaining undelivered women, cervical score increased from 3.1 to 6.0 after PGE₂ gel treatment, and from 3.8 to 4.7 after placebo gel. This difference is statistically significant ($p < 0.01$).

In the subsequent open study, 70 women with varying parity received 0.5 mg PGE₂ gel intracervically. Thirty-eight out of these (54 %) were induced into labor and delivered without further stimulation within 24 hours.

In the undelivered women, an increase in cervical score from 3.2 to 6.5 was observed within 24 hours.

No caesarean sections had to be undertaken. Four women (8%) were delivered by ventouse due to signs of fetal distress during late second stage of labor.

The impact on labor induction of intracervically applied PGE₂ gel, related to gestational age in patients with an unripe cervix (II)

In this study 54 term pregnant women participated. Group A, included 27 nulliparous and group B, 27 parous women. The gestational age ranged between 38 and 42 weeks. All women received 0.5 mg PGE₂ gel intracervically.

In group A, 18 women (66 %) with a mean initial cervical score of 3.3 and in group B, 13 women (38 %) with a mean initial cervical score of 3.7 were delivered within 24 hours. The gestational age was almost identical in the delivered and undelivered patients. The original cervical score was, however, lower in women undelivered after 24 hours.

The total number of instrumental deliveries in group A was 7 out of 27 (26 %). They consisted of 3 caesarean sections and 4 ventouses. The frequency of instrumental deliveries was not related to gestational age, but 3 out of 4 women who had to be delivered by ventouse had a cervical score < 3.

In group B, two women were delivered by caesarean section and one with ventouse corresponding to 11 %. Two of these women had cervical score < 3.

Induction of term labor in women with unripe cervix
combining intracervical application of PGE₂ gel with
intravenous infusion of oxytocin (III)

To investigate the appropriate time to obtain cervical priming after PGE₂ application, and to find out whether the procedure could be combined with intravenous infusion of oxytocin, 54 pregnant women with an unripe cervix received 0.5 mg PGE₂ in gel intracervically. Their cervical states were checked at regular intervals (Fig. 1). After 5 hours, 37 women of 54 (70 %) had favorable cervical states and seven were in active labor. Fifteen of the remaining 30 women had favorable cervical states (mean improvement of cervical score from 3.6 to 7.0). They received randomly at that time intravenous infusion of oxytocin. Thirteen were delivered within 12 hours. The other 15 women with a mean improvement of cervical score from 3.5 to 6.8 were randomized for intravenous oxytocin the next morning, i.e., 24 hours after application of the PGE₂ gel. Seven of these 15 women went, however, without further stimulation into labor during the night and were delivered before the next morning. The remaining 8 women received intravenous oxytocin and were delivered within 12 hours. Ten women out of the 54 still had unfavorable cervical states 24 hours after the gel application. These women received another intracervical application of 0.5 mg PGE₂ in gel. In 6, favorable cervical states were registered 5 hours later. Four women still had unfavorable cervixes even after the second gel application. Two of

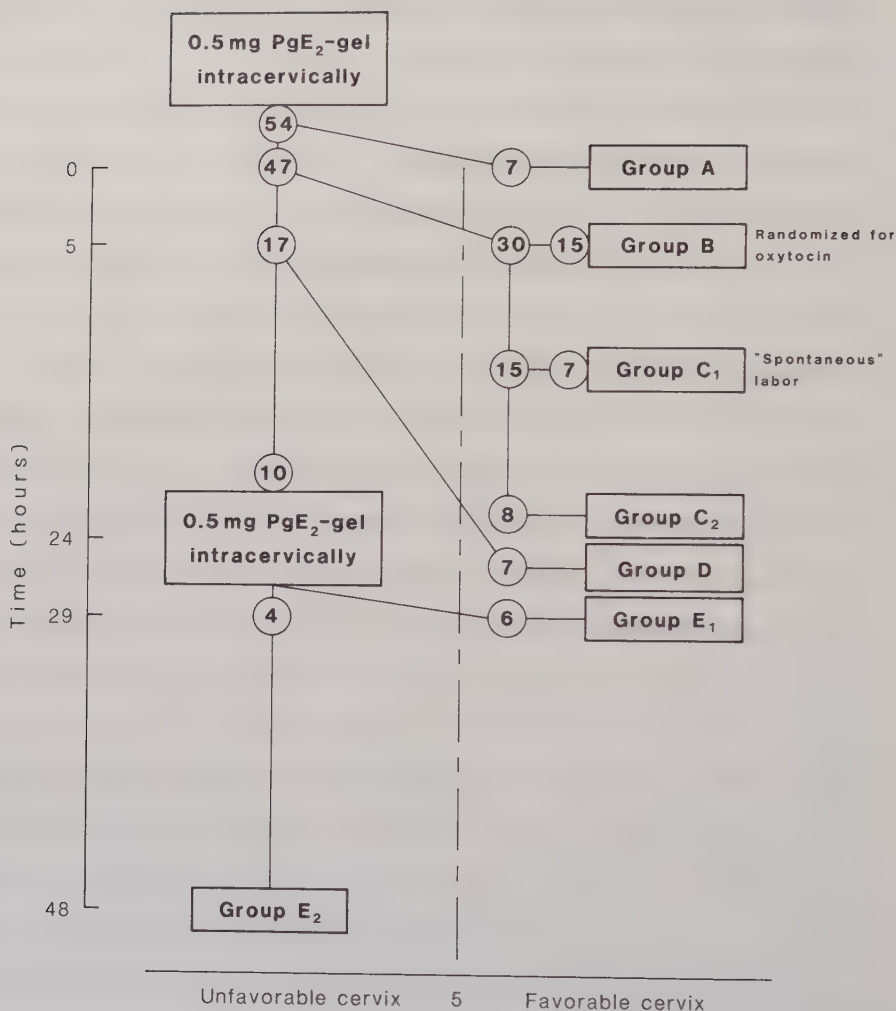


Fig. 1 Cervical ripening after intracervical application of 0.5 mg PGE₂-gel in fifty-four women with unripe cervix at term.

them had to be delivered by caesarean sections. Hence, induction of labor failed in only two out of 54 women (4 %). The total number of instrumental deliveries was 10 (20 %), including 4 caesarean sections (7 %).

Intravaginal versus intracervical application of prostaglandin E₂ in gel for cervical priming or term labor induction in patients with an unfavorable cervical state
(IV)

To compare the effect of intravaginal and intracervical PGE₂ gel, 60 term pregnant women, (30 nulliparous, group A and 30 multiparous, group B), all with unfavorable cervical states were randomly treated with PGE₂ in viscous gel in a dose of 0.5 mg intracervically or 4 mg intravaginally. In order to quantitate the myometrial activity after the gel-treatment, intrauterine pressure (IUP) was recorded by microtransducers in eight women (4 after intracervical and 4 after intravaginal gel application).

In group A, including nulliparous women, 12 out of 15 had favorable cervical states 5 hours after intracervical application and 10 of them were delivered within 12 hours. Among the 15 nulliparous women who received 4 mg PGE₂ gel intravaginally, 4 had favorable cervical states after 5 hours and were delivered within 12 hours. These differences are statistically significant ($p < 0.05$).

In group B, all the 15 multiparous women had favorable cervical states after intracervical application, and 12 were delivered within 12 hours. After intravaginal gel

treatment, 7 of 15 women had favorable cervical states after 5 hours and were delivered within 12 hours. The difference between the two groups concerning the number of successful priming within 5 hours is statistically significant ($p < 0.01$).

If the results are analysed in women ($n = 35$) with highly unfavorable cervixes, i.e., initial score ≤ 3 , the difference between the two modes of treatment is highly significant concerning cervical priming (83 % after intracervical and 6 % after intravaginal application respectively) ($p < 0.001$) and in nulliparous women also concerning labor induction (67 % after intracervical compared to 0 % after intravaginal treatment) ($p < 0.01$).

No side effects were registered after intracervical application, but three women had attacks of vomiting after the intravaginal route of application. Furthermore, three women had painful contractions during cervical priming after the intravaginal treatment.

When the IUP recordings were analysed, it was found that intracervical application increased myometrial activity, but only to a small degree, whereas a considerable increase was seen after intravaginal PGE_2 gel application. There was a significant difference in the myometrial activity expressed in Montevideo Units between the two

modes of treatment already 30 minutes after application of the gel, i.e., 70 versus 15 Montevideo Units, ($p < 0.001$).

Due to signs of fetal distress, caecarean section had to be performed in one woman after intracervical and in four women after intravaginal application.

Spontaneous cervical ripening related to changes in collagen, glycosaminoglycans and collagenolytic activity
(V)

Cervical biopsies were obtained from 55 women: 15 non-pregnant, 22 early-pregnant, 7 term-pregnant and 11 vaginally delivered within 5 to 10 minutes. The DNP-peptide hydrolytic activity (collagenase) and the concentrations of elastase, collagen, sulphated glycosaminoglycans, hyaluronic acid, and water were estimated. A gradual decrease in the collagen concentration (hydroxyproline) and a corresponding increase in the collagenolytic activity (DNP-peptide hydrolytic activity and leucocyte elastase) were found during progress of pregnancy and parturition. Also the physiochemical properties of the collagen changed, as expressed by a higher extractability in acetic acid with pepsin, during both pregnancy and labor compared to the nonpregnant state.

Significantly lower concentration of sulphated glycosaminoglycans and hyaluronic acid was found in term and post partum biopsies compared to that found in cervix from non- and early-pregnant women ($p < 0.01$). The water content was

significantly higher late in pregnancy and after delivery than in the non-pregnant state ($p < 0.001$). The concentration of collagen in cervical biopsies taken after delivery was well correlated to the cervical dilatation time, i.e., the lower the collagen concentration, the shorter the time of labor.

Collagenolytic activity post partum in cervical connective tissue after treatment with prostaglandin E_2 (VI)

To investigate the change in the biochemical composition of cervical connective tissue after PGE_2 induced priming, biopsies were taken from 7 women induced into labor after intracervical application of 0.5 mg PGE_2 in gel. Cervical specimens from 11 spontaneously delivered women served as controls.

The specimens were analysed for the DNP-peptide hydrolytic activity (collagenase) and the concentrations of leucocyte elastase, collagen, sulphated glycosaminoglycans, hyaluronic acid, and water. Five hours after application of the PGE_2 gel, favorable cervical states were found in 6 out of 7 women. Four of them had regular uterine contractions and two women were at that time given intravenous infusion of oxytocin to induce or to augment labor. The remaining woman who still had an unripe cervix 5 hours after the gel application, started labor without further stimulation about 9 hours later.

At admission, all 11 women in the control group had had effective uterine contractions for 2 to 3 hours and had a cervical dilatation of 2 - 3 cm.

In the cervical biopsies taken immediately after delivery, the DNP-peptide hydrolytic activity (collagenase) was significantly higher in the PGE₂ treated women than in the controls, i.e., 550 U/100 mg and 372 U/100 mg, respectively ($p < 0.05$). In the four women with a prompt response to the PGE₂ treatment, the collagenase activity was highly increased compared to the controls, i.e., 688 U/100 mg versus 372 U/100 mg ($p < 0.01$). However, the concentration of hydroxyproline (collagen) in the PGE₂ treated patients was not significantly lower than in the controls, i.e., 6.2 µg/mg and 6.7 µg/mg wet weight, respectively. Concerning the concentrations of sulphated glycosaminoglycans, hyaluronic acid, water, and leucocyte elastase, no significant differences were found between the two groups of women.

None of the patients in any of these investigations (I-VI) had any serious side effects and all children were born in good condition with 5 minutes Apgar score > 7 .

The new gel technique for intracervical application of PGE_2 using a crosslinked starch powder as a vehicle was found effective both to induce cervical ripening and to induce term labor in women with an unfavorable cervix.

The results of the double blind study confirmed that the PGE_2 compound, and not the gel vehicle or the application procedure per se, is responsible for the ripening. Furthermore, the results of the open study were similar to those obtained in previous investigations using conventional gels both concerning the frequency of induced labor and the successful cervical priming (Ulmsten and Wingerup 1979). Most important is also the observation that the high efficacy was obtained without serious adverse effects. This confirms that intracervical application of 0.5 mg PGE_2 in gel is a safe and effective method for cervical priming and for labor induction in this category of patients.

Unlike conventional cellulose based gels, the new PGE_2 gel preparation has the advantage that it can be industrially produced, thus complying with demands of homogeneity, stability and sterility (Ulmsten and Wingerup 1979). Moreover, the consistency of the gel can be modified by adjusting the quantity of added saline. Thus, a small gel volume with a firm and sticky consistency makes a more

strict intracervical application possible compared to conventional gel preparations. This is of importance both from practical and scientific aspects. By using a "concentrated" PGE_2 gel for intracervical application, it was shown that the myometrial stimulation during cervical ripening can be minimized (Forman et al. 1982a,b). In the present investigation (IV), this was also confirmed. Similarly, the number of women induced into labor after a single application of 0.5 mg PGE_2 in a small gel volume was lower (III) than after application of a similar amount PGE_2 in a larger gel volume (26 % and 54 % respectively) (Ulmsten et al. 1980).

Even if it is obvious that myometrial stimulation combined with a decrease in cervical resistance should be an optimal technique for evacuation of the pregnant uterus, it might be advantageous in several term pregnant women to obtain cervical priming without prominent myometrial activity. It is then very interesting to note that cervical priming without considerably increased myometrial activity was achieved within 5 hours in the majority of women in study III. Another important observation was that intravenous infusion of oxytocin then could be administered to induce labor without the occurrence of myometrial hyperstimulation. Such a two-step procedure implies new possibilities for term labor induction in high risk pregnancies. As shown in study III, the majority of the women could be delivered vaginally without complications

within 12 hours after start of the treatment - a most encouraging result in view of the problematic category of patients induced.

Also worth noting is the result of study II, emphasizing that, although not disregarding parity and gestational age, the cervical state seems to be the most important factor in predicting the outcome of the inductive trial. Hence, the the importance of a proper assessment of cervical state before labor induction must be stressed.

The vaginal route of prostaglandin application has been considered most attractive, as being easy to use, non-invasive, and offering the possibility for self-administration. Good effects with high frequency of successful cervical priming and labor induction have also been reported in several investigations (MacKenzie and Embrey 1977, Davey et al. 1980, Embrey et al. 1980, Shepherd et al. 1979, Khoo et al. 1981). Hitherto no prospective comparative studies on intracervical and intravaginal PGE₂ gel administration seem to exist. The results from the present investigation showed that in women with a moderately unripe cervix, there were small differences in efficacy between the two routes of application. However, in women with a highly unfavorable cervix (≤ 3 points), the intracervical route was found significantly more effective compared to the intravaginal, both concerning cervical priming and labor induction.

Due to the prompt absorption of the prostaglandin compound into systemic circulation after intravaginal application, this route has been claimed to be just another form of systemic administration (Embrey 1975, Gordon-Wright and Elder 1979b). This view is also supported by the present results, showing a significantly increased myometrial activity after intravaginal application compared to intracervical. Similarly, gastro-intestinal discomfort was registered after vaginal, but not after intracervical, PGE₂ gel treatment.

Also worth consideration is the observation that, despite the obvious stimulatory effects on the myometrium after intravaginal application, and the almost complete lack of such effects after intracervical PGE₂ gel application, cervical ripening was more effective following the latter procedure. This supports previous observations that priming of the cervix is not primarily or entirely dependent on uterine contractions, but also on reactions occurring within the cervical tissue itself (Forman et al. 1982a, Forman et al. 1982b).

In this context, the results of the experimental studies (V and VI) are essential contributions to the understanding of spontaneous and pharmacologically induced cervical priming. Earlier data concerning biochemical changes within the human cervix are inconsistent (von Maillot et al. 1979, Kitamura et al. 1979, Cabrol et al. 1980, Shimizu et al. 1981). In the present study we are showing

that clinically registered cervical priming can be related to prominent biochemical changes in the cervical connective tissue. These changes concern the concentrations of collagen, acid mucopolysaccharides, and water.

It is not surprising that the prominent effects in both spontaneous and PGE_2 induced cervical ripening are associated with an increase of the collagenolytic activity. This suggests a degradation of the dominating supporting structure of the human cervix - the collagen fibrils. The cervical ripening is also combined with a change in the physicochemical properties of the collagen indicated by an increased extractability in acetic acid with pepsin. In this respect, the results of study V are in agreement with the observations made by Ito et al. (1979a), and by von Maillot et al. (1976). Most likely, the change in the solubility of the collagen is an expression for the remodulation of the cervical connective tissue. Since there was no further decrease in collagen concentration during parturition, the remodulation process seems to be more or less completed at the onset of labor (Kleissl et al. 1978).

A most important observation was that collagen concentration in cervical connective tissue was well correlated to the cervical dilatation time during parturition. Consequently, estimation of collagen concentration in cervix uteri before induction of labor would be of great value as

an objective parameter predicting the inducibility of the woman. These data stress the importance of a favorable cervix for the outcome of labor.

For our collagenase determinations we used DNP-peptide as substrate (Masui et al. 1977). However, the major part of cervical collagenase is inactivated by bounding to α_2 -macroglobulin. This inhibition might be a result of the extraction procedure. Nevertheless, the DNP-peptide determines the total activity of the enzyme including both free and inhibited collagenase. It seems therefore to be a proper substrate for measuring collagenase activity (Masui et al. 1977, Kitamura et al. 1980a).

Liggins (1981) has described cervical ripening as a modified inflammatory reaction. The gradual increase in the concentration of leucocyte elastase during normal cervical ripening supports this concept. It is also in line with the findings of Junqueira et al. (1980) who described a dissolution of connective tissue occurring around leucocytes in the uterine cervix after delivery. Not only collagenase and leucocyte elastase are involved in the degradation of collagen during cervical priming, but also other enzymes, e.g., neutral proteinases, PZ - peptidase and cathepsin D. The importance of these enzymes remains, however, to be further investigated (Ito et al. 1979b, Ito et al. 1980, Hutchins et al. 1981, Dingle 1981).

Since the PGE_2 induced cervical ripening clinically mimics spontaneous cervical ripening, it is logic that we found the collagenolytic activity to be increased in cervical connective tissue after local PGE_2 treatment (VI). These findings support the in vitro results published by Conrad and Ueland (1979) who, using a strain stretch model, found a decreased resistance in cervical tissue specimens from patients delivered after PGE_2 induction compared to those from spontaneously delivered.

Furthermore, we found that the collagenolytic activity significantly increased in women with a prompt response to the PGE_2 treatment compared to those with a moderate effect. Despite the high collagenolytic activity after PGE_2 treatment, the collagen concentration was not significantly lower compared to that found in spontaneously delivered women. The explanation for this could be that the degraded collagen is retained within the tissue for some time as suggested by Kleissl et al. (1978).

Conclusively, both spontaneous and PGE_2 -induced cervical ripening are associated with pronounced changes in the cervical connective tissue. In a small number of patients, the unripe cervix seem, however, to be resistant to repeated PGE_2 treatment. This could indicate that also other hormones, i.e., relaxin or oestrogen, are of importance for the ripening process (Stewart et al. 1981, McLennan et al. 1981, Anderson et al. 1982).

CONCLUSIONS

1. Intracervical application of 0.5 mg PGE_2 in a viscous gel based on a crosslinked starch polymer is effective to produce cervical ripening and to induce labor in patients with an unfavorable cervix at term. The obtained effect is due to the PGE_2 component and not to the viscous medium or the application procedure.

2. The outcome of PGE_2 -gel treatment is primarily related to the original cervical state. Parity and gestational age have less influence.

3. In 70 % of patients receiving 0.5 mg PGE_2 gel intracervically, a favorable cervical state is achieved within 5 hours after gel application. Intravenous infusion of oxytocin can then be instituted to induce or augment labor.

4. In patients with a highly unfavorable cervix, intracervical application is superior to intravaginal, whereas in patients with a "moderately" unripe cervix, the two routes of PGE_2 gel treatment seem to be equipotent. Side effects are more likely to occur after intravaginal gel application. The myometrial response to the gel treatment is significantly more increased after intravaginal application compared to intracervical.

5. Spontaneous cervical ripening during pregnancy and parturition is associated with prominent biochemical changes in the cervical connective tissue. These changes are a pronounced degradation of collagen most likely caused by a nearly ten-fold increase of the collagenolytic activity (DNP-peptide hydrolytic activity and elastase) and a significant decrease in the concentrations of proteoglycans.

Pharmacologically induced cervical ripening (intracervical application of 0.5 mg PGE₂ gel) is associated with similar biochemical changes as those seen in spontaneous ripening.

REFERENCES

- Anderson, A. B. M. & Turnbull, A. C. 1969. Relationship between length of gestation and cervical dilatation, uterine contractility and other factors during pregnancy. *Am J Obstet Gynecol* 105:1207.
- Andersson, K.-E., Forman, A. & Ulmsten, U. 1982. Pharmacology of labor. In: *Clin Obstet Gynecol* (ed. K. Ueland and U. Ulmsten), in press. Lippincott company.
- Antonopoulos, C. A., Gardell, S., Szirmai, J. A. & De Tyssonsk, E. R. 1964. Determinations of glycosaminoglycans (mucopolysaccharides) from tissues on the microgram scale. *Biochim Biophys Acta* 83:1.
- Bengtsson, L. P. 1968. Cervical insufficiency. *Acta Obstet Gynecol Scand Suppl* 1.
- Bishop, E. H. 1964. Pelvic scoring for elective induction. *Obstet Gynecol* 24:266.
- Brummer, H. C. 1971. Storage life of prostaglandin E₂ in ethanol and saline. *J Pharm Pharmacol* 23:804.
- Bygdeman, M. 1978. Comparison of prostaglandin and hypertonic saline for termination of pregnancy. *Obstet Gynecol* 52:424.
- Bygdeman, M. 1980. Clinical applications. *Advances in prostaglandin and thromboxane research*. Vol 6 (ed. B. Samuelsson et al), p. 87. Raven Press, New York.
- Cabrol, D., Breton, M., Berrou, E., Visser, A., Sureau, C. & Picard, J. 1980. Variations in the distribution of glycosaminoglycans in the uterine cervix of the pregnant woman. *Eur J Obstet Gynecol Reprod Biol* 10:281.
- Calder, A. A., Embrey, M. P. & Tait, T. 1977. Ripening of the cervix with extra-amniotic prostaglandin E₂ in viscous gel before induction of labor. *Br J Obstet Gynaecol* 84:264.
- Calder, A. A. 1979. The management of the unripe cervix. In: *Human parturition*. First ed. (ed. M. Keirse et al), p. 201. Leiden University Press.
- Campbell, S. & Newman, G. B. 1971. Growth of the fetal biparietal diameter during normal pregnancy. *J Obstet Gynaecol Br Cwlth* 78:513.
- Caplan, A. I. & Hascall, V. 1980. Structure and developmental changes in proteoglycans. In: *Dilatation of the uterine cervix* (ed. F. Naftolin and P. G. Stubblefield), p. 79. Raven Press, New York.

Carlson, Don M. 1968. Structures and immunochemical properties of oligosaccharides isolated from pig submaxillary mucines. *J Biol Chem* 243:616.

Conrad, J. T. & Ueland, K. 1979. The stretch modulus of human cervical tissue in spontaneous, oxytocin-induced and prostaglandin E_2 -induced labor. *Am J Obstet Gynecol* 133:11.

Danforth, D. N. 1947. The fibrous nature of the human cervix and its relation to the isthmic segment in gravid and nongravid uteri. *Am J Obstet Gynecol* 53:541.

Danforth, D. N. 1954. The distribution and functional activity of the cervical musculature. *Am J Obstet Gynecol* 68:1261.

Danforth, D. N., Veis, A., Breen, M., Weinstein, H. G., Buckingham, J. C. & Manalo, P. 1974. The effect of pregnancy and labor on the human cervix: Changes in collagen glycoproteins and glycosaminoglycans. *Am J Obstet Gynecol* 120:641.

Davey, D. A., Domisse, J. & MacNab, M. 1980. Intravaginal prostaglandin E_2 for cervical ripening and induction of labour. *South African Med J* 58:516.

Dingle, J. T. 1981. A possible role for catabolin in tissue remodelling and repair. In: *The fetus and independent life* (CIBA Foundation Symposium No. 86), p. 203. Pitman, London.

Embrey, M. P. 1970. Induction of labour with prostaglandins E_1 and E_2 . *Br Med J* 2:256.

Embrey, M. P. 1975. The prostaglandins in human reproduction, p. 48. Edinburg, Churchill-Livingstone.

Embrey, M. P., Graham, N.B. & McNeill, M.E. 1980. Induction of labour with a sustained-release prostaglandin E_2 vaginal pessary. *Br Med J* 281:901.

Forman, A., Ulmsten, U., Wingerup, L. & Bányai, J. 1982a. Effects of intracervical PGE_2 -gel on myometrial activity and cervical state in first trimester pregnancy. *Prostaglandins* 24:303.

Forman, A., Ulmsten, U., Bányai, J., Wingerup, L. & Uldbjerg, N. 1982b. Evidence for local effect of intracervical PGE_2 -gel. *Am J Obstet Gynecol*. In press.

Garett, W. J. 1960. Prognostic signs in surgical induction of labour. *Med J Aust* 49:929.

Gillespie, A. 1972. Prostaglandin-oxytocin enhancement and potentiation and their clinical applications. *Br Med J* 1:150.

Gillespie, A., Brummer, H. C. & Chard, T. 1972. Oxytocin release by infused prostaglandin. *Br Med J* 1:543.

Gordon-Wright, A. P. & Elder, M. G. 1979a. Prostaglandin E_2 tablets used intravaginally for the induction of labour. *Br J Obstet Gynaecol* 86:32.

Gordon-Wright, A. P. & Elder, M. G. 1979b. The systemic absorption from the vagina of prostaglandin E_2 administered for the induction of labour. *Prostaglandins* 18:153.

Gustavii, B. 1975. Release of lysosomal acid phosphatase into the cytoplasm of decidual cells before the onset of labour in humans. *Br J Obstet Gynaecol* 82:177.

Hendricks, C. H., Brenner, W. E. & Kraus, G. 1970. Normal cervical dilatation pattern in late pregnancy and labor. *Am J Obstet Gynecol* 106:1065.

Hutchins, C. J. & Parkin, E. N. 1981. Pz-peptidase activity in the pregnant and non-pregnant human cervix. *Br J Obstet Gynaecol* 88:150.

Ito, A., Kitamura, K., Mori, Y. & Hirakawa, S. 1979a. The change in solubility of type 1 collagen in human uterine cervix in pregnancy at term. *Biochem Med* 21:262.

Ito, A., Mori, Y. & Hirakawa, S. 1979b. Purification and characterization of an acid proteinase from human uterine cervix. *Chem Pharm Bull* 27:969.

Ito, A., Ihara, H. & Mori, Y. 1980. Partial purification and characterization of a novel neutral proteinase from human uterine cervix. *Biochem J* 185:443.

Junqueira, L. C. U., Zugaib, M., Montes, G. S., Toledo, O. M. S., Krisztán, R. M. & Shigihara, K. M. 1980. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilatation. *Am J Obstet Gynecol* 138:273.

Karim, S. M. M., Devlin, J. & Hillier, K. 1968. The stability of dilute solutions of prostaglandin E_1 , E_2 , F_1 , and F_2 . *Eur J Pharmacol* 4:416.

Karim, S. M. M. & Sharma, S. D. 1971. Oral administration of prostaglandins for the induction of labour. *Br Med J* 1:260.

Karim, S. M. M., NG, S. C. & Ratnam, S. S. 1980. Termination of abnormal intrauterine pregnancy with prostaglandins. Singapore J Obstet Gynecol 11:7.

Karube, H., Kanke, Y., Mori, Y., Hirakawa, S. & Hayashi, M. 1975. Increase of structural glycoproteins during dilatation of the human cervix in pregnancy at term. Endocrinol Japon 22:445.

Khoo, P. P. T., Kalshekar, M., Jogee, M. & Elder, M. G. 1981. Induction of labour with prostaglandin E₂ vaginal tablets. Eur J Obstet Gynaecol Reprod Biol 11:313.

Kitamura, K., Ito, A., Mori, Y. & Hirakawa, S. 1979. Changes in the human uterine cervical collagenase with special reference to cervical ripening. Biochem Med 22:332.

Kitamura, K., Ito, A. & Mori, Y. 1980a. The existing forms of collagenase in the human uterine cervix. J Biochem 87:753.

Kitamura, K., Ito, A., Mori, Y. & Hirakawa, S. 1980b. Glycosaminoglycans of human uterine cervix: Heparan sulfate increase with reference to cervical ripening. Biochem Med 23:159.

Kleissl, H. P., Van der Rest, M., Naftolin, F., Glorieux, F. H. & De Leon, A. 1978. Collagen changes in the human uterine cervix at parturition. Am J Obstet Gynecol 113:748.

Krapohl, A. J., Myers, G. G. & Caldeyro-Barcia, R. 1970. Uterine contractions in spontaneous labor. Am J Obstet Gynecol 106:378.

Leppert, P. C., Keller, S., Cerreta, J. & Mandl, I. 1982. Conclusive evidence for the presence of elastin in human and monkey cervix. Am J Obstet Gynecol 142:179.

Liggins, G. C., Forster, C. S., Grieves, S. A. & Schwartz, A. L. 1977. Control of parturition in man. Biol Reprod 16:39.

Liggins, G. C. 1981. Cervical ripening as an inflammatory reaction. In: The cervix in pregnancy and labour (ed. D. A. Ellwood and A. B. M. Anderson), p.1. Churchill-Livingstone.

Lindahl, U. & Höök, M. 1978. Glycosaminoglycans and their binding to biological macromolecules. Ann Rev Biochem 47:385.

Mac Kenzie, I. Z. & Embrey, M. P. 1977. Cervical ripening with intravaginal prostaglandin E₂ gel. Br Med J 2:1381.

Mac Kenzie, I. Z. & Embrey, M. P. 1979. A comparison of PGE₂ and PGF_{2α} vaginally gel for ripening of cervix before induction of labour. Br J Obstet Gynaecol 86:167.

Mac Lennan, A. H., Green, R. C., Bryant-Greenwood, G. D., Greenwood, F. C. & Seamark, R. F. 1980. Ripening of the human cervix and induction of labour with purified porcine relaxin. Lancet 2:220.

Masui, Y., Takemoto, T., Sakakibara, S., Hori, H. & Nagai, Y. 1977. Synthetic substrates for vertebrate collagenase. Biochem Med 17:215.

Mitchell, M. D. 1981. Prostaglandins during pregnancy and the perinatal period. J Reprod Fert 62:305.

Najak, Z. & Hillier, K. 1970. The action of prostaglandins on the human isolated non pregnant cervix. J Obstet Gynaecol Br Cwlth 77:701.

Nakaya, T. 1973. Studies on acid mucopolysaccharides in the human cervix uteri. Nagoya Med J 18:295.

Ohlsson, K. & Olsson, A.-S. 1978. Immunoreactive granulocyte elastase in human serum. Hoppe-Seyler's Z Physiol Chem 359:1531.

Ohlsson, K. 1980. Polymorphonuclear leucocyte collagenase. In: Collagenase in normal and pathological connective tissues (ed. D. E. Woolley and J. M. Evanson), p. 209. John Wiley & Sons Ltd.

Persson, P. H., Grennert, L., Gennser, G. & Gullberg, B. 1978. Normal range curves for the intrauterine growth of biparietal diameter. Acta Obstet Gynecol Scand Suppl 78:15.

Rorie, D. K. & Newton, M. 1967. Histologic and chemical studies of the smooth muscle in the human cervix and uterus. Am J Obstet Gynecol 99:466.

Roseman, T. J., Sims, B. & Stehle, R. G. 1973. Stability of prostaglandins. Am J Hosp Pharm 30:236.

Schwalm, H. & Dubrauszky, V. 1966. The structure of the musculature of the human uterus - muscles and connective tissue. Am J Obstet Gynecol 94:391.

Scott, J. E. & Orford, C. R. 1981. Dermatan sulphate-rich proteoglycan associates with rat tail-tendon collagen at the d-band in the gap region. Biochem J 197:213.

Shepherd, J., Sims, C. & Craft, I. 1976. Extra amniotic prostaglandin E₂ and the unfavourable cervix. Lancet 2:709.

Shepherd, J. H., Benett, M. J., Laurence, D., Moore, F. & Sims, C. D. 1981. Prostaglandin vaginal suppositories: A simple and safe approach to the induction of labor. *Obstet Gynecol* 58:596.

Shimizu, T., Endo, M. & Yosizawa, Z. 1980. Glycoconjugates (glycosaminoglycans and glycoproteins) and glycogen in the human cervix uteri. *Tohoku J Exp Med* 131:289.

Srivastava, K. C. & Clausen, J. 1973. Stability of prostaglandin E compounds in solution. *Lipids* 8:592.

Stegeman, H. & Stalder, K. 1967. Determination of hydroxyproline. *Clin Chim Acta* 18:267.

Stewart, P., Kennedy, J. H., Barlow, D. H. & Calder, A. A. 1981. A comparison of oestradiol and prostaglandin E₂ for ripening the cervix. *Br J Obstet Gynaecol* 88:236.

Thiery, M., Defoort, P., Benijts, G., Van Eyck, J., Hennay, T., Van Kets, H. & Martens, G. 1977. Effectiveness of extra-ovular injection of prostaglandin E₂ in tylose gel to ripen the cervix prior to elective induction of labor at term. *Prostaglandins* 14:381.

Thiery, M. 1979. Induction of labor with prostaglandins. In: *Human Parturition* (ed. M. J. N. C. Keirse et al), p. 155. Leiden University Press.

Turnbull, A. C. & Andersson, A. B. M. 1968. Induction of labour. Part II. *J Obstet Gynaecol Br Cwlth* 75:24.

Uldbjerg, N., Malmström, A., Ekman, G., Sheehan, J., Ulmsten, U. & Wingerup, L.: 1982a. Dermatan sulphate proteoglycan from human uterine cervix: Isolation and characterization. Submitted.

Uldbjerg, N., Karlstedt, I., Ekman, G., Malmström, A., Ulmsten, U. & Wingerup, L. 1982b. Dermatan sulphate and mucinglycopeptides from the human uterine cervix. Submitted.

Ulmsten, U. & Andersson, K.-E. 1979. Multichannel intra-uterine pressure recording by means of micro-transducers. *Acta Obstet Gynecol Scand* 58:115.

Ulmsten, U., Andersson, K.-E., Lindström, K. & Persson, P. H. 1979. Determination of myometrial tension during labor by combined microtransducer IUP-recording and ultrasonic examination of the uterine cavity. *Acta Obstet Gynecol Scand* 58:547.

Ulmsten, U. & Wingerup, L. 1979. Cervical ripening induced by prostaglandin E₂ in viscous gel. *Acta Obstet Gynecol Scand Suppl* 84.

Ulmsten, U. & Wingerup, L. 1980. Clinical experiences with a new gel for intracervical application of prostaglandin E₂ before therapeutic abortion or induction of term labor. *Prostaglandins* 20:533.

Van der Rest, M. 1980. Collagen and its metabolism. In: *Dilatation of cervix* (ed. F. Naftolin and P. G. Stubblefield), p. 61. Raven Press.

Von Maillot, K. & Zimmermann, B. K. 1976. The solubility of collagen of the uterine cervix during pregnancy of labour. *Arch Gynecol* 220:275.

Von Maillot, K., Stuhlsatz, H. W., Mohanaradhakrishnan, V. & Greiling, H. 1979. Changes in the glycosaminoglycans distribution pattern in the human uterine cervix during pregnancy and labor. *Am J Obstet Gynecol* 135:503.

Vorherr, H. 1975. Placental insufficiency in relation to post term pregnancy and fetal postmaturity. *Am J Obstet Gynecol* 123:67.

Wolley, D. E. & Evanson, J. M. 1981. *Collagenase in normal and pathological connective tissues*. Chichester, New York, Brisbane, Toronto. John Wiley and Sons.

Öbrink, B. 1973. A study of the interactions between monomeric tropocollagen and glycosaminoglycans. *J Biochem* 33:387.

INTRACERVICAL APPLICATION OF PROSTAGLANDIN
GEL FOR INDUCTION OF TERM LABOR

U. Ulmsten, L. Wingerup, P. Belfrage,
G. Ekman and N. Wiquist

From the Department of Obstetrics and Gynaecology at General Hospital, Malmö, Karolinska Hospital, Stockholm, and Sahlgrens Hospital, Gothenburg, Sweden.

ABSTRACT

A new gel for local application of prostaglandins has been elaborated. The new gel, based on a lyophilized prostaglandin E_2 (PGE_2) starch powder, seems to have solved most of the pharmaceutical and clinical problems associated with local administration of prostaglandins. In a randomized double-blind study, 50 nulliparous patients with an unfavorable cervical state at term were given 2 ml gel containing 0.5 mg PGE_2 (PGE_2 gel) or gel without PGE_2 (placebo gel). The gel was deposited into the cervical canal. Among patients given PGE_2 gel, 11 of 25 had induced labor, delivering without further stimulation within 24 hours. In patients given placebo gel, 2 of 25 were delivered within 24 hours. This difference is statistically significant ($P < .01$). Patients undelivered after treatment with PGE_2 gel achieved a considerable and statistically significant improvement of cervical score, whereas in patients in whom labor was not induced successfully by placebo gel treatment no significant changes in cervical score were registered. In a subsequent open study another 70 term patients of varied parity were given 0.5 mg PGE_2 gel. Thirty-eight patients (54%) had successfully induced labor. Among the remaining undelivered patients, considerable ripening of the cervix occurred. Thus, the cervical score changed from a mean of 3.2 prior to treatment to a mean of 6.5 by 24 hours after treatment. Gastrointestinal discomforts were not observed. Signs of uterine hyperstimulation were registered in 1 patient.

Prostaglandins are generally characterized as locally effective hormones. Applied as drugs, they should therefore be located as closely to the target organ as possible to increase their efficacy and reduce their side effects. By using different cellulose-based gels (hypromellose and tylose), prostaglandin E_2 (PGE_2) has been applied extra-amniotically, intracervically or intravaginally, either to prime the unfavorable cervix, or to induce term labor, or both.¹⁻⁵ Although promising results have been reported, it has been obvious that the described ex tempore gel preparations have several disadvantages. Thus, bacterial contamination and inhomogeneity of the prepared gel combined with the instability of the PGE_2 decreasing the amount of active substance can jeopardize the outcome of treatment. Demands for more standardized gel formulations have been raised by pharmacologists and pharmaceutical manufacturers.⁶⁻⁹

Recently the authors elaborated a new gel for local application of prostaglandins based on a special cross-linked starch polymer.^{10,11} Initial clinical experiences with this gel were promising^{12,13} and prompted the undertaking of a more extensive study to test the effectiveness and safety of the new PGE_2 gel technique in the clinical routine. This article describes the authors' experiences from a 3-center study using the new PGE_2 gel to prime the cervix, or to induce labor in patients with an unfavorable cervical state at term, or both.

PATIENTS AND METHODS

Patients

The study comprised 2 groups of patients treated at 3 different medical centers. The first group constituted a randomized double-blind study in which 50 women were given a single dose of 2 ml gel containing 0.5 mg PGE₂ (PGE₂ gel) or 2 ml without PGE₂ (placebo gel). All patients were nulliparas with a mean age of 25 years (range, 18 to 34 years). Mean gestational age was 41 weeks (range, 38 to 43 weeks). As shown in Table 1, characteristics of patients receiving PGE₂ gel were comparable with those of patients receiving placebo gel.

A consecutive open study consisted of 70 patients with varied parity given PGE₂ gel of the same dose administered in the double-blind study. Table 1 presents relevant data on the patients in this group. In all patients labor had to be induced for medical or strong psychosocial reasons. The most common indications for induction of labor were postmaturity (gestational age greater than 42 weeks) and preeclampsia. All women were screened by ultrasound to exclude those with breech presentation, multiple pregnancy, or low-implanted placenta. The study was approved by the ethics committees at the 3 separate hospitals.

Preparation of the gel

As the gel preparation has been described in detail

Table 1. Clinical Data (Mean $\pm$ SD)

Factor	Double-blind study		Open study	
	Placebo group	PGE ₂ group	Nulliparous women	Parous women
	(N = 25)	(N = 25)	(N = 35)	(N = 35)
Patient age (yr)	25.5 $\pm$ 4.1	25.3 $\pm$ 4.6	27.1 $\pm$ 4.5	30.3 $\pm$ 4.6
Gestational age (wk)	41.7 $\pm$ 1.1	41.0 $\pm$ 1.5	40.7 $\pm$ 1.3	39.7 $\pm$ 1.6
Cervical score before treatment	3.7 $\pm$ 1.1	3.5 $\pm$ 1.3	3.5 $\pm$ 1.1	3.7 $\pm$ 1.2

PGE₂ = prostaglandin E₂.

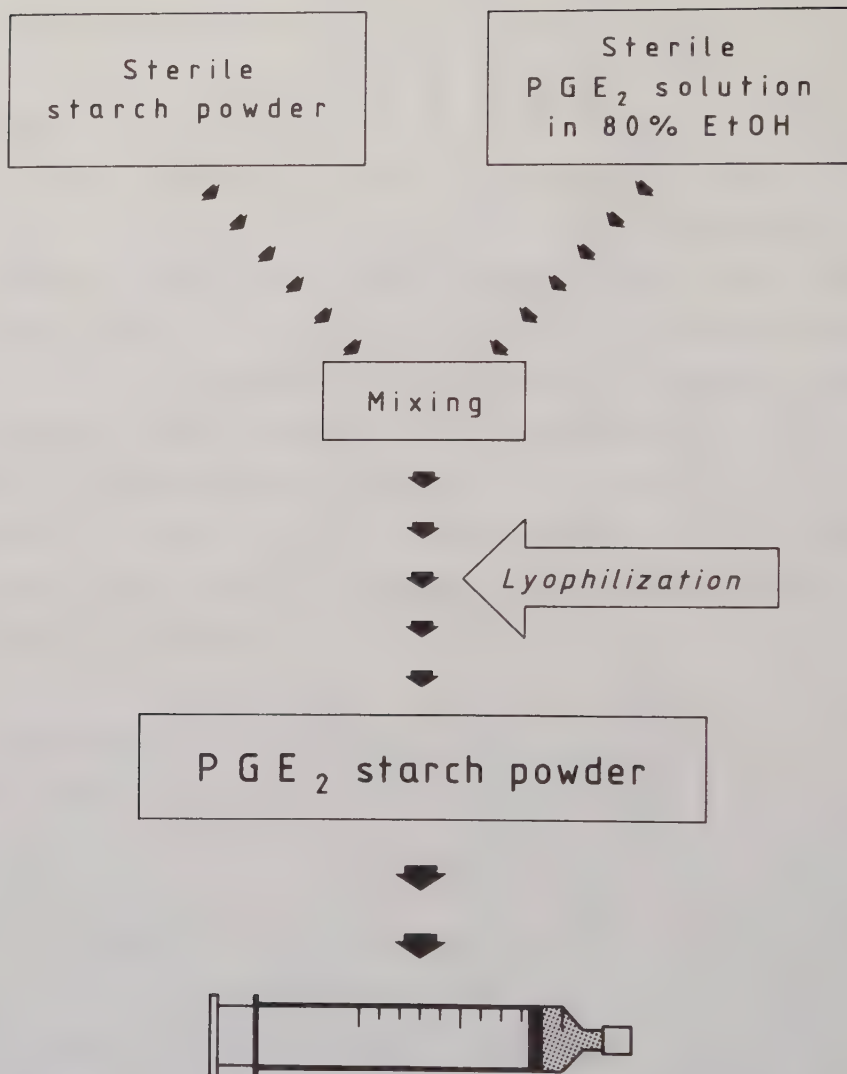


Fig. 1 Schematic drawing of the principal steps in the production of PGE₂ starch powder.

Table 2. Modified Bishop Score Used in Present Study

Factor	Score		
	0	1	2
Cervical dilatation	< 0.5 cm	0.5 - 1.5 cm	> 1.5 cm
Cervical effacement	None	< 50 %	> 50 %
Station of fetal head	Above or in pelvic inlet	Above spinae	At or below spinae
Cervical consistency	Firm	Medium	Soft
Cervical position	Posterior	Mid	Anterior

elsewhere,¹² it is only briefly outlined here. As shown in Figure 1, PGE₂ in 80% ethanol solution (Upjohn) is mixed with a special cross-linked starch polymer (Perstorp AB, Sweden). The solution is lyophilized, and portions of the resulting PGE₂ starch powder are dispensed in 5-ml plastic syringes, each containing 0.5 mg PGE₂. The procedure described here is performed industrially under aseptic conditions. Analyses have demonstrated that the PGE₂ powder can be stored for more than 1 year without a significant decrease in the activity of the PGE₂ component.¹¹ Before clinical use, the obstetrician adds only 2 ml of physiologic saline to the syringe; within 15 seconds, the powder swells to a ready-to-use homogeneous gel. The amount of PGE₂ can be varied on request. Moreover, by varying the amount of saline added, gel viscosity can be adjusted.

Management of patients

Before application of the gel, the cervical state was assessed according to a modification of Bishop's original scoring system most commonly used throughout Scandinavia (Table 2). To enhance the validity of the cervical recordings, all obstetricians involved in the study practiced cervical assessment as a team to attain complete conformity.

The PGE₂ gel was applied within the cervical canal using a 12-cm-long, 3-mm-thick nylon catheter with 2 side holes

0.5 cm from the tip. At application, the authors attempted to deposit all of the gel within the cervical canal starting at the level of the internal os. Because of the physical characteristics of the gel, particularly its high viscosity, leakage into the vagina was minimized.¹¹ Immediately after gel application, the catheter was removed. From 15 to 30 minutes before to 1 hour after gel application, patients were monitored by external cardiotocography. Patients in whom no abnormalities were registered and labor failed to be induced were referred to the antenatal care unit of the department. Those in whom labor was established were supervised according to the routine of the department, which included external or internal cardiotocography; scalp blood samples were taken when indicated. Amniotomy was not performed until the cervix was dilated 4 cm or more and labor was well established. Oxytocin or other stimulating agents were not infused.

Patients in whom labor was not induced were reexamined the next morning, ie, 24 hours after gel application. This examination included a reassessment of the cervical state.

RESULTS

The outcome of the gel treatment is summarized in Table 3. As indicated, 11 of 25 patients receiving PGE₂ gel had induced labor and delivered within 24 hours; only 2 of 25 patients in the placebo-treated group responded similarly.

This difference is statistically significant ($P < .01$). Among the patients undelivered after PGE_2 gel treatment, cervical score increased from 3.1 to 6.0. In patients treated with placebo gel only a slight increase from a mean of 3.8 to 4.7 was seen. This difference is statistically significant ($P < .01$).

In the subsequent open study, in which all patients were treated with 0.5 mg PGE_2 gel, the same favorable result was obtained as in the patients receiving PGE_2 gel in the double-blind trial. Thus, 38 of 70 (54%) patients had labor induced and delivered within 24 hours. The remaining undelivered 32 patients showed an increase in cervical score from a mean of 3.2 to 6.5, recorded within the same period (Table 3); 27 (84%) of these women had labor successfully induced and delivered within 24 hours after oxytocin infusion.

Mean induction-to-delivery time among the patients in the double-blind study delivered after PGE_2 gel treatment was 9.5 hours (range, 6.0 to 16.5 hours). In patients delivered after PGE_2 gel treatment in the open study, mean induction-to-delivery time was 10.5 hours (range, 4.0 to 23.0 hours).

Maternal gastrointestinal discomfort, hyperpyrexia, and signs of infection were not observed. In 1 patient of 95 given 0.5 mg PGE_2 signs of uterine hypercontractility

Table 3. Results of Treatment

	Double-blind study		Open study	
	Placebo group (N = 25)	PGE ₂ group (N = 25)	Nulliparous women (N = 35)	Parous women (N = 35)
Delivered within 24 hours	2 (8%)	11 (44%)	15 (43%)	23 (66%)
Undelivered	23	14	20	12
Cervix score before treatment	3.8 $\pm$ 1.1	3.1 $\pm$ 1.5	3.4 $\pm$ 0.9	2.9 $\pm$ 1.2
Cervix score after treatment	4.7 $\pm$ 1.6	6.0 $\pm$ 1.9 [†]	6.7 $\pm$ 1.4	6.3 $\pm$ 1.7

PGE₂ = prostaglandin E₂.

* P < .01, Fisher exact test.

† P < .01, Wilcoxon rank-sum test, 1-tailed.

occurred; too strong and too frequent uterine contractions were observed approximately 6 hours after gel application, culminating 7 hours later, at which point transient fetal bradycardia appeared. Intravenous administration of 0.5 mg terbutaline alleviated uterine hypercontractility, and the fetal heart rate resumed a normal pattern. The patients was delivered 23 hours after PGE₂ gel application, and the infant had an Apgar score of 10 after 1 minute.

No cesarean section had to be undertaken. Four patients (8%) were delivered by ventouse. The primary indication for instrumental delivery was signs of fetal distress during late second stage of labor. All children were delivered in good condition with a 5-minute Apgar score of 7 or higher.

DISCUSSION

The present results confirm those previously attained with the new PGE₂ gel formulation.^{12,13} They are also consonant with those reported after treatment with conventional cellulose-based gels.^{1,4,5,14} Furthermore, pharmaceutically and pharmacologically, the new gel technique holds considerable advantages over former techniques. The new gel can be produced industrially on a large scale under controlled aseptic conditions without deterioration in homogeneity and stability. Hence, the precise amount of active PGE₂ in each gel portion is guaranteed.¹¹ Moreover,

the new gel preparation is easily handled by the obstetrician. As the dose of prostaglandin and the viscosity of the gel can be varied, the new gel technique will also facilitate laboratory study.

As in previous double-blind studies,^{4,12,15} the present investigation verifies that it is the PGE_2 component, rather than the gel vehicle per se or its application, that is responsible for the ripening of the cervix.

It has been suggested that prostaglandins might induce cervical priming independent of uterine contractions.^{16,17} The records from the present investigation cannot be used for proper separation of myometrial and local cervical effects on the process of cervical priming. Consonant with previous reports,^{14,15,18} however, the authors frequently observed prominent cervical ripening as well in patients not exhibiting signs or symptoms of increased myometrial activity after PGE_2 gel application.

Recent studies have indicated that cervical priming by PGE_2 gel is associated with marked changes in the ultrastructure of the cervical fibrous stroma.¹⁹ Thus, a considerable increase in the amount of glycosaminoglycans - a major component of the ground substance - is recorded. The total water content remains unchanged. Concomitantly with the biochemical changes, prominent histologic changes are seen. Collagen fibers are dissolved and split up

clearly because of an increase in the amount of ground substance.¹⁹ These have been observed in patients treated with the new PGE₂ gel technique. For reasons given previously, above all because of the standardization of the treatment, the present PGE₂ gel formulation seems to be most convenient for investigations of the effects of prostaglandins on the intact human cervix.

Probably because of the low dose of PGE₂ used and its intracervical application, the priming effect on the cervix obtained in the present study was associated with a low frequency of side effects. The same positive results have been found using the gel technique in young nulliparous women prior to termination of pregnancy by dilatation and evacuation late in the first trimester.^{12,13} Moreover, results from ongoing studies indicate that the new gel can also be used for intravaginal application of prostaglandins. It might therefore be justifiable to conclude that the new PGE₂ gel technique will facilitate the management of pregnancies with an unfavorable cervical state, both at induction of term labor and before termination of pregnancy by dilatation and evacuation.

REFERENCES

1. Calder, A.A., Embrey, M.P. & Tait, T.: Ripening of the cervix with extraamniotic prostaglandin E_2 in viscous gel before induction of labour. *Br J Obstet Gynaecol* 84:264, 1977.
2. MacKenzie, I.Z., Embrey, M.P.: Cervical ripening with intravaginal prostaglandin E_2 gel. *Br Med J* 2:1381, 1977.
3. Thiery, M., Defoort, P., Benijts, G., et al.: Effectiveness of extraovular injection of prostaglandin E_2 in Tylose[®] gel to ripen the cervix prior to elective induction of labour at term. *Prostaglandins* 14:381, 1977.
4. Wingerup, L., Andersson, K.-E., & Ulmsten, U.: Ripening of the uterine cervix and induction of labour at term with prostaglandin E_2 in viscous gel. *Acta Obstet Gynecol Scand* 57:403, 1978.
5. Ulmsten, U. & Wingerup, L.: Cervical ripening induced by prostaglandin E_2 in viscous gel. *Acta Obstet Gynecol Scand (Suppl)* 84:11, 1979.
6. Elder, M.G. & Gordon-Wright, A.P.: Prostaglandins for cervical ripening. *Lancet* 1:779, 1979.
7. Lippert, T.H.: The use of prostaglandin gel in obstetrics and gynecology. *Arch Gynaekol* 227:171, 1979.
8. Moore, F.G.: Prostaglandins and cervical ripening. *Lancet* 1:501, 1979.
9. Editorial: The unripe cervix. *Lancet* 1:364, 1979.
10. Ulmsten, U., Johansson, O., Kirstein-Pedersen, A., et al.: New gel for intracervical application of prostaglandin E_2 . *Lancet* 1:377, 1979.
11. Harris, A.S., Kirstein-Pedersen, A., Stenberg, P., et al.: The preparation, characterization and stability of new prostaglandin E_2 gel for local administration. *J Pharm Sci* 69:1271, 1980.
12. Ulmsten, U., Kirstein-Pedersen, A., Stenberg, P., et al.: A new gel for intracervical application of prostaglandin E_2 . *Acta Obstet Gynecol Scand (Suppl)* 84:19, 1979.
13. Ulmsten, U. & Wingerup, L.: Clinical experiences with a new gel for intracervical application of prostaglandin E_2 before therapeutic abortion or induction of term labour. *Prostaglandins* 20:533, 1980.
14. Ulmsten, U., Wingerup, L. & Andersson, K.-E.: Comparison of prostaglandin E_2 and intravenous oxytocin for induction of labor. *Obstet Gynecol* 54:581, 1979.
15. Wingerup, L., Ulmsten, U. & Andersson, K.-E.: Ripening of the cervix by intracervical application PGE₂-gel before termination of pregnancy with dilatation and evacuation. *Acta Obstet Gynecol Scand (Suppl)* 84:15, 1979.
16. Liggins, G.C.: Prostaglandins: Current therapeutic status in obstetrics. *Drugs* 8:161, 1974.
17. Liggins, G.C.: Ripening of the cervix. *Semin Perinatol* 2:261, 1978.

18. Stys, S.J., Clewell, W.H. & Meschia, G.: Changes in cervical compliance at parturition independent of uterine activity. *Am J Obstet Gynecol* 130:414, 1978.
19. Uldbjerg, N., Ekman, G., Malmström, A., et al.: Biochemical and morphological changes of human cervix after local application of prostaglandin E₂ in pregnancy. *Lancet* 1:267, 1981.

THE IMPACT ON LABOR INDUCTION OF INTRACERVICALLY
APPLIED PGE₂-GEL, RELATED TO GESTATIONAL AGE
IN PATIENTS WITH AN UNRIPE CERVIX

G. Ekman, P.-H. Persson, U. Ulmsten, L. Wingerup

From the Department of Obstetrics and Gynecology, Malmö General Hospital, University of Lund, Malmö, Sweden, and the Department of Obstetrics and Gynecology, University of Århus, Århus, Denmark

ABSTRACT

In 54 patients with an unripe cervix in late third trimester, in which gestational age had been properly determined by repeated ultrasound scannings, labor was induced by intracervical application of 0.5 mg PGE₂ in viscous gel. It was found that the outcome of the induced labor was not related to the gestational duration, but to the pre-inductive cervical score. Thus, the number of successful inductions was smaller and induction delivery time longer, the lower the cervical score. Moreover, instrumental deliveries occurred most frequently in nulliparous women, with a low pre-inductive cervical score. Taking into consideration the difficulties of labor induction in the present type of patient, the overall proportion of instrumental deliveries, (17 %, including 7 % caesarean sections) was low. No maternal or fetal side effects were observed. It is concluded that intracervical application of a small dose of PGE₂ in gel can be recommended for cervical priming and labor induction in pre- and post-term pregnancy.

It is generally accepted that gestational age affects the outcome of induced labor (3,5,9). However, to be able to study the relationship between gestational age and inducibility an exact determination of gestational age is essential. In previously reported studies on this subject, no precise methods for the proper determination of gestational age seem to have been used.

In our department, all pregnant women are examined twice with ultrasound, in the 17th and 32nd weeks of pregnancy. The fetal biparietal diameter (BPD) is measured ad modum Campbell (1). The reproducibility of our BPD measurements has been carefully investigated and the total error in the determination of gestational age has been calculated not to exceed ± 5 days in 90 % of the pregnancies (4).

With these excellent possibilities for correct estimation of gestational age we were interested to study if labor induction and cervical priming with prostaglandin E_2 (PGE_2) in gel was more complicated and less successful in women with an unripe cervix before and after term, compared with that in patients on exact time of expected delivery.

MATERIAL AND METHODS

Patients

Fifty-four pregnant women around term participated in a

consecutive study from February 1, 1980 to February 1, 1981. All were informed about the purpose of the study and gave their consent. All had an unfavorable cervical state (modified Bishop score ($\leq$ 5) (10). In all patients the indications for induction were obstetric, i.e. toxaemia, fetal growth retardation, isoimmunization, hepatitis, suspected placental insufficiency, thrombosis, and diabetes.

According to parity, the patients were allocated to two groups. Group A consisted of 27 nulliparous women with a mean age of 24.5 years (24 - 34). Their gestational ages ranged from the 38th to the 43rd week and mean cervical score was 3.0 (range 1 - 5). Group B consisted of 27 parous women with a mean age of 31.2 years (24 - 39). Their gestational ages were 38th - 43rd week and mean cervical score was 3.2 (range 1 - 5).

All patients had head presentations and intact membranes. Patients with a low implanted placenta and patients with multiple pregnancy were excluded from the investigation.

Two routine ultrasound scannings performed, in the 17th and 32nd week of pregnancy, enabled a proper estimation of gestational age to be made, as earlier described.

Preparation and application of the gel

PGE₂ in 80% ethanol solution (Upjohn) is mixed with a

special cross-linked starch polymer (Perstorp AB, Sweden). The solution is lyophilized and portions of the resulting PGE₂ starch powder are dispensed in 5-ml plastic syringes, each portion containing 0.5 mg of PGE₂. Before clinical use, the obstetrician adds 2.5 ml of physiological saline into the syringe and within 15 sec the powder swells to a ready-to-use homogenous gel (2-6).

Before application of the gel, the cervical state was assessed according to a modified Bishop score (10).

With the patient in semilithotomy position, 0.5 mg PGE₂-gel was applied into the cervical canal using a 12 cm long and 3.3 mm thick nylon catheter with two side holes 0.5 cm from the tip. The gel application started at the level of the internal os and the gel was then slowly infused during continuous withdrawal of the catheter. After completion of the gel application, the catheter was removed. Supervision of the patients was carried out by external cardiotocography (Hewlett Packard). No further stimulation was performed and amniotomy was not undertaken until labor was well established and the cervix dilated to $\geq$ 4 cm. If labor was not induced, the cervical score was reassessed the next morning, i.e. 24 hours after gel application, whereafter conventional intravenous infusion of oxytocin was instituted.

RESULTS

In Group A, 18 women (66 %) with a mean cervical score of 3.4 and in Group B, 13 women (48 %) with a mean cervical score of 3.7 gave birth within 24 hours after a single PGE₂-gel application (see Table I). This table also shows that gestational age was almost identical in the delivered and undelivered patients.

Table II shows gestational week, induction delivery time (IDT) and pre-inductive cervical score in the women who gave birth within 24 hours. As most easily recognized in patients in Group A, the number of successful inductions was smaller and IDT longer, the lower the cervical score. Moreover, instrumental deliveries occurred most frequently in nulliparous patients with low pre-inductive cervical scores.

In patients undelivered after 24 hours a considerable improvement in cervical state was recorded. Thus, mean cervical score improved from 3.0 (range 1 - 5) to mean 6.4 (range 6 - 8) in Group A and in Group B from mean 3.3 (range 2 - 5) to mean 6.7 (range 6 - 8) 24 hours after PGE₂-gel application. The degree of priming was not related to gestational age. In all undelivered patients, labor could be induced with infusion of oxytocin the day after PGE₂-gel application.

Table I. Results of PGE₂-induction in 54 patients with unripe cervix.

	Patients' age (years)	Gestational age (weeks)	Cervix score before treatment
<u>Nulliparous women (Group A)</u>			
<u>n</u> = 27			
Delivered $\leq$ 24 hours, <u>n</u> = 18 (66%)	25.2 (range 18-32)	39.8	3.4 (range 2-5)
Undelivered $\leq$ 24 hours, <u>n</u> = 9 (44%)	23.1 (range 18-39)	40.0	2.6 (range 1-5)
<u>Parous women (Group B)</u>			
<u>n</u> = 27			
Delivered $\leq$ 24 hours, <u>n</u> = 13 (48%)	29.7 (range 25-34)	39.8	3.7 (range 2-5)
Undelivered $\leq$ 24 hours, <u>n</u> = 14 (52%)	31.2 (range 24-39)	40.0	2.9 (range 1-5)

Table II. Data on 18 nulliparous and 13 parous women delivered ≤ 24 hours after application of 0.5 mg PGE₂-gel intracervically.

Gestational age (weeks)	Total number of patients	Number of delivered patients	Cervix score before treatment	Induction delivery time (hours)
<u>Group A</u>				
Preterm ≤ 38	13	9 ^{••**}	3.1	14.4
Term 40	6	4 [•]	4.3	8.2
Post term ≥ 42	8	5 [•]	3.8	11.0
Total number	27	18 (66%)	3.4	10.7
<u>Group B</u>				
Preterm ≤ 38	11	5 [•]	4.0	9.4
Term 40	7	2 [•]	2.3	12.8
Post term ≥ 41	9	6 [•]	4.4	6.5
Total number	27	13 (48%)	3.7	10.5

• Delivered by ventouse

•• Delivered by caesarean section

The total number of instrumental deliveries in Group A was 6 out of 27 (22 %) representing 2 caesarean sections and 4 ventouses. All patients delivered by caesarean section had an original cervical score < 3 . Moreover, 3 out of 4 patients delivered by ventouse had a cervical score < 3 . The indications for caesarean section were signs of fetal distress in one patient and in the other, failure of labor to progress.

In Group B, 2 women were delivered by caesarean section and one with ventouse corresponding to 11 % of the whole group. Two out of the 3 patients delivered instrumentally in this group had a cervical score < 3 . The indications for caesarean section were signs of fetal distress in one patient and in the other, failure of labor to progress.

Irrespective of the mode of delivery, all infants were born in good condition, with an Apgar score < 7 within 5 min. No maternal side effects were noticed. Thus, no patient complained of gastrointestinal discomfort and no signs of hyperpyrexia or intra-uterine infections were observed.

COMMENTS

The unripe cervix in patients at term presents the obstetrician with a well-known clinical problem. As has been shown previously, intracervical application of a small

dose of PGE_2 primes the cervix effectively and induces labor in approximately 50 % of term patients, without further stimulation (7,8,10). Though the number of patients in the present study was small, the results indicate that the outcome of induction in late third-trimester pregnancy seems to be more related closely to pre-inductive cervical state than to gestational duration. Moreover, the priming effect of PGE_2 -gel on the unfavorable cervix seems to be just as good in preterm and post-term patients as in those at term.

The importance of the pre-inductive cervical state for the outcome of induced labor is further emphasized by the findings that IDT was longest and instrumental deliveries most frequent in patients with the lowest cervical scores. Taking into consideration the well-known difficulties of labor induction of patients similar to those in our study, the total number of instrumental deliveries 17 % (including 7 % caesarean sections) must be considered quite acceptable.

Intracervical application of PGE_2 in gel can therefore be recommended for cervical priming and labor induction in pre- and post-term patients with an unripe cervix. The effectiveness of the procedure related to the absence of maternal and fetal side effects makes it an attractive method for labor induction in these types of patients.

REFERENCES

1. Campbell, S.: An improved method of fetal cephalometry by ultrasound. *J Obstet Gynaecol Br Commonw* 75: 568, 1968.
2. Harris, A.S., Kirstein-Pedersen, A., Stenberg, P., Ulmsten, U. & Wingerup, L.: Preparation, characterization and stability of a new prostaglandin E₂-gel for local administration. *J Pharm Sci* 69: 1271, 1980.
3. Hendricks, C.H., Brenner, W.E. & Kraus, G.: Normal cervical dilatation pattern in late pregnancy and labor. *Am J Obstet Gynecol* 106: 1065, 1970.
4. Persson, P.H., Grennert, L., Gennser, G. & Gullberg, B.: Normal range curves for the intrauterine growth of biparietal diameter. *Acta Obstet Gynecol Scand, Suppl.* 78: 15, 1978.
5. Turnbull, A.C. & Anderson, A.B.M.: Induction of labour. Part II. Intravenous oxytocin infusion. *J Obstet Gynaecol Br Commonw* 75: 24, 1968.
6. Ulmsten, U., Kirstein-Pedersen, A., Stenberg, P. & Wingerup, L.: A new gel for intracervical application of prostaglandin E₂. *Acta Obstet Gynecol Scand, Suppl.* 84: 19, 1979.
7. Ulmsten, U. & Wingerup, L.: Clinical experiences with a new gel for intracervical application of prostaglandin E₂ before therapeutic abortion or induction of term labor. *Prostaglandins* 20: 533, 1980.
8. Ulmsten, U., Wingerup, L., Belfrage, P., Ekman, G., Floberg, J., Karlsson, K. & Wikqvist, N.: A new gel technique for local application of prostaglandins to prime the unfavourable cervix at induction of term labour. A three center study. *Obstet Gynecol* 59: 336, 1982.
9. Vorherr, H.: Placental insufficiency in relation to postterm pregnancy and fetal postmaturity. *Am J Obstet Gynecol* 123: 67, 1975.
10. Wingerup, L., Andersson, K.-E. & Ulmsten, U.: Ripening of the uterine cervix and induction of labor at term with prostaglandin E₂ in viscous gel. *Acta Obstet Gynecol Scand* 57: 403, 1978.

INTRACERVICAL APPLICATION OF PGE_2 -GEL COMBINED WITH EARLY
INTRAVENOUS INFUSION OF OXYTOCIN FOR INDUCTION OF TERM
LABOR IN WOMEN WITH UNRIPE CERVIX

Gunvor Ekman, Ulf Ulmsten and Lars Wingerup

Department of Obstetrics and Gynecology, Malmö General Hospital, University of Lund, Sweden, Department of Obstetrics and Gynecology, University of Århus, Århus, Denmark

ABSTRACT

Fifty-four women with unripe cervix at term were given 0.5 mg prostaglandin E_2 (PGE_2) in gel intracervically. In thirty-seven (70 %) a favorable cervical state was achieved after 5 hours. In seven of these women labor was established at that time. Of the remaining thirty patients fifteen were randomly given intravenous infusion of oxytocin. By that therapy thirteen were delivered within twelve hours. No side-effects of the combined treatment were registered.

In seventeen women (30 %) the cervix was still unfavorable 5 hours after PGE_2 -gel application. Without further therapy seven of these women had a favorable cervical state when reassessed the next morning, i.e. 24 hours after PGE_2 -gel application. Without complication all these patients were induced into labor with intravenous infusion of oxytocin. The remaining ten patients had to receive another PGE_2 -gel application with subsequent intravenous infusion of oxytocin to be induced into labor. In two patients, the induction failed and these patients had to be delivered by Caesarean section. Irrespective of treatment and mode of delivery all children were born in good condition.

It is concluded that intracervical application of prostaglandin E_2 followed by intravenous infusion of oxytocin is an effective and safe method for labor induction in patients with unfavorable cervix at term.

In several investigations, including both double blind studies and comparisons with oxytocin, prostaglandin E_2 (PGE_2) applied extra-amniotically or intracervically in gel has been found an effective agent for ripening the unfavorable cervix at term.^{1,8,11,13,14,16} As yet, no close subsequent induction or augmentation of labor with intravenous oxytocin has been performed after the gel-application. One reason for this has been the possible risk of myometrial hyperstimulation by adding oxytocin to the prostaglandin-treatment.^{5,6,12} Another the lack of prospective studies penetrating the time taken for cervical priming to occur after PGE_2 -gel application. Earlier observations indicated cervical priming to occur already 4 - 6 hours after PGE_2 -gel application (Ulmsten 1980, unpublished data). The present investigation was undertaken to determine if ripening of the cervix could be obtained shortly i.e. within 5 hours after gel-application and if intravenous infusion of oxytocin then could be used to induce or augment labor.

MATERIAL AND METHOD

Fifty-four pregnant women all with an unfavorable cervix participated in the study. Thirty-two were nulliparous with a mean age of 26.5 years (range 18 - 34) and a mean gestational age of 41.2 weeks (range 38 - 43); 22 were parous with a mean age of 30.0 years (range 26 - 42), a mean parity of 1.5 (range I - III) and a mean gestational

age of 40.1 weeks (range 38 - 42). All subjects were informed about the purpose of the study and the procedures involved and gave their consent.

The indications for induction were all obstetric, i.e., toxemia, fetal growth retardation, hepatosis, isoimmunization and suspected placental insufficiency. All patients had head presentations and intact membranes. Two ultrasound scannings in weeks 17 - 18 and 32 - 33 respectively enabled proper estimation of gestational age and excluded multiple pregnancy and low implanted placenta.

Apart from a few minor changes, i.e., the volume of added saline was reduced to 2 ml giving increased viscosity, the gel was prepared and applied as described in detail earlier.^{7,13,15} Moreover, considerable effort was made to apply the gel strictly within the cervical canal. Thus, application started at the internal os and the gel was instilled during a slow continuous withdrawal of the catheter. As in previous studies, each portion of gel contained 0.5 mg PGE₂.

Before gel application, the cervical state was assessed according to a modified Bishop score.¹³ For practical reasons, the gel was applied early in the morning and cervical state was then reassessed in all undelivered patients after 5, 24, 29 and 48 hours.

Half the number of patients who had achieved a favorable cervical state (cervical score > 5) 5 hours after gel application was randomly given intravenous infusion of oxytocin (Group B, Fig. 1). In the other half, no further treatment was given until next morning, i.e., 24 hours after gel application. At that time intravenous infusion of oxytocin was instituted according to the schedule outlined below (Group C₂, Fig. 1).

Patients with an unfavorable cervical state 5 hours after gel application were reexamined after 24 hours. If cervix then was ripen (cervical score > 5), labor was induced by intravenous infusion of oxytocin (Group D, Fig. 1). In case of a still unripe cervix, another intracervical application of 0.5 mg PGE₂ in gel was given. If the cervix after that second gel application was favorable after 5 hours, intravenous infusion of oxytocin was instituted (Group E₁, Fig. 1). Patients with still unripe cervix the subsequent morning i.e. 48 hours after the initial gel application had infusion of oxytocin and if no success, these patients were delivered by Caesarean section (Group E₂, Fig. 1).

Oxytocin was administered intravenously using an electronic infusion pump. The infusion started with 2 mU/min and was subsequently increased by 2 mU/min every half hour up to 24 mU/min. Amniotomy was not undertaken until labor was well established and cervix dilated to ≥ 4 cm.

All patients were supervised according to the routines of the delivery ward. Thus external cardiotocography (Hewlett-Packard) was performed from 1 hour before to at least 1 hour after gel application. In case of induced myometrial activity the monitoring continued until the patient was delivered or uterine contractions ceased. Internal cardiotocography was instituted immediately after rupture of the membranes. pH measurements from the fetal scalp was taken when indicated.

Myometrial hypocontractility was defined as: uterine contractions occurring more frequently than 5 per 10 minutes; a contraction amplitude exceeding 60 mm Hg; resting pressure between contractions exceeding 15 - 20 mm Hg.

RESULTS

The outcome of the treatment was the same for nulliparous and parous patients. In the following, nulliparous and parous patients will therefore be dealt with together. As can be seen from Fig. 1 five groups of patients (A, B, C, D and E) could be distinguished according to outcome of the treatment. Fourteen women (26 % of the total material) with an initial mean cervical score of 3.1 were delivered within 24 hours after a single intracervical application of 0.5 mg PGE₂-gel (Group A, and C₁, Fig. 1). Five hours after gel application, seven of these 14 women had a

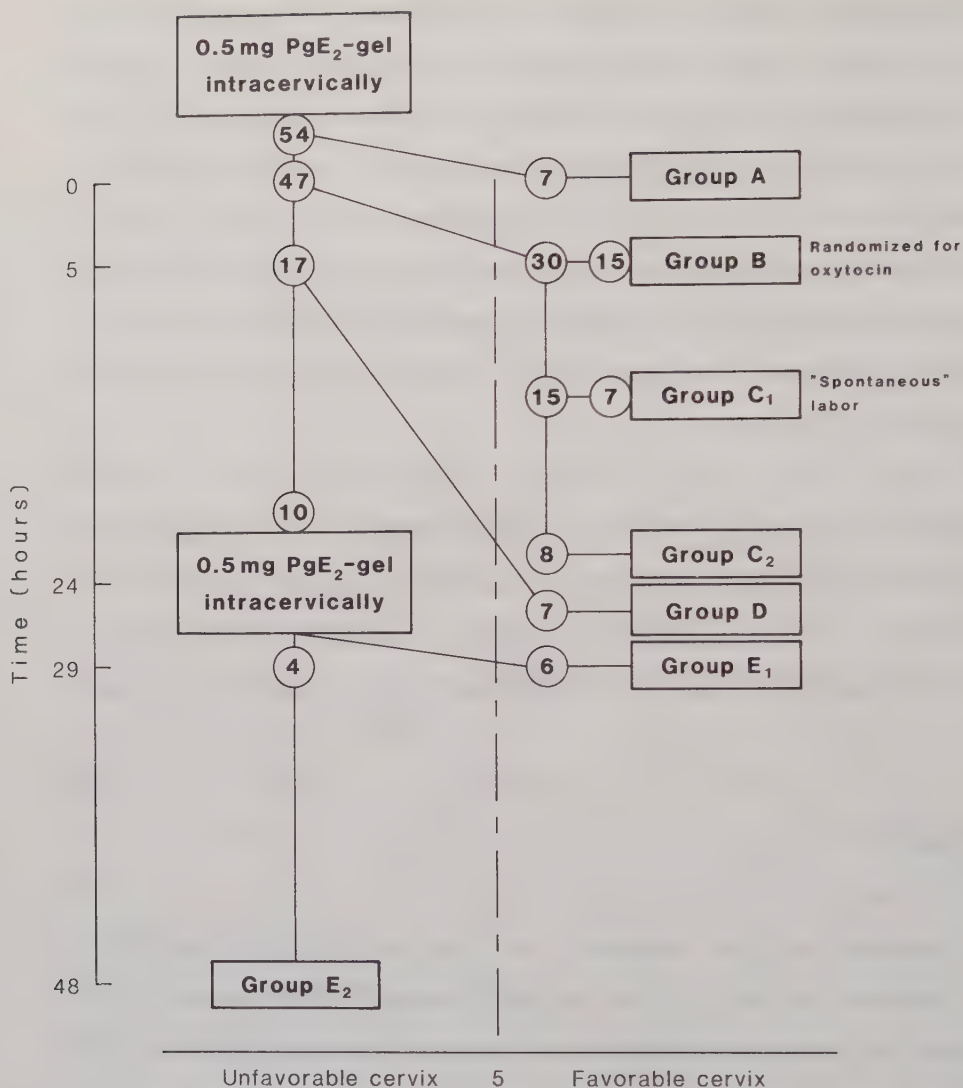


Fig. 1 Cervical ripening after intracervical application of 0.5 mg PGE₂-gel in fifty-four women with unripe cervix at term.

favorable cervical state and inconsistent uterine contractions while the remaining seven were in labor with effective regular uterine contractions.

In 30 women (55 % of the total material) the cervix was primed 5 hours after gel application without registration of concomitant uterine activity. Fifteen of these patients, with a mean improvement in cervical score from 3.6 to 7.0, were then randomly given intravenous oxytocin and thirteen (87 %) were delivered within 12 hours (Group B, Fig. 1). Among the other 15 patients with a mean improvement of cervical score from 3.5 to 6.8 who not received oxytocin, seven went into labor and were delivered without further stimulation within 24 hours (Group C₁, Fig. 1). The cervical score of the remaining eight patients was not found further improved at reexamination next morning, i.e., 24 hours after the initial gel application. All but two of these eight women were delivered within 12 hours after labor induction by oxytocin infusion.

Seven women with a mean cervical score of 3.6 before gel treatment had improved their cervical score to mean 4.4 five hours after gel application (Group D). When reexamined the following morning, an improvement of cervical score to a mean of 7.7 was registered and all were delivered within 12 hours after the start of oxytocin infusion.

Ten women (Group E, Fig. 1) with an initial highly unfavorable cervical score (mean 1.8) still had an unfavorable cervical state at examination 5 and 24 hours after gel application (cervical score 3.2 and 3.8 respectively). In these women a second gel application of 0.5 mg PGE₂ was carried out 24 hours after the first one. Within 5 hours, six women had a favorable cervix. Four were delivered within 10 hours and two within 24 hours after start of oxytocin infusion (Group E₁, Fig. 1). The remaining four women (Group E₂) were all nulliparous with a mean initial cervical score of 1.5. They still had an unfavorable cervical state after two gel applications (mean cervical score 3.6). Two of the patients were delivered vaginally after a second infusion of intravenous oxytocin, whereas the remaining two had to be delivered by Caesarean section due to failure of labor to progress. Thus, in only 2 out of 54 patients (4 %) induction of labor failed.

The total number of instrumental deliveries was 10 (20 %) including 4 Caesarean sections (7 %). The reason for the Caesarean sections were in two patients signs of fetal distress in second stage of labor; in one subject during labor induced by PGE-gel application only (Group A), in the other during infusion of oxytocin 24 hours after PGE₂-gel application (Group C). The indication for induction in these two patients were suspect placental insufficiency.

The other two patients subjected to Caesarean section were those of group E₂ in whom labor failed as described above. The indication for labor induction in these patients was toxemia and Mb. Crohn respectively.

No maternal side effects were registered irrespective of the mode of treatment. Thus no patient complained of gastrointestinal discomfort and no signs of myometrial hypercontractility were observed. Irrespective of the mode of treatment and delivery all children were born in good condition with Apgar score > 7 after 5 minutes.

DISCUSSION

The present investigation did not involve any control groups since recently performed double-blind studies have confirmed the PGE₂-component and not the gel vehicle or the administration procedure to induce the cervical priming.^{16,17} Moreover, comparative studies with intracervical PGE₂-gel and intravenous infusion of oxytocin have shown that in patients with an unripe cervix myometrial stimulation with oxytocin induces no significant improvement of cervical score compared to that found after PGE₂-gel treatment.^{4,14}

In the present investigation 26 % of the patients were induced into labor and delivered within 24 hours after a single intracervical application of 0.5 mg PGE₂ in gel.

This figure is lower than that reported in previous investigations.^{13,16} Moreover, compared to previous studies the gel-application did not induce uterine contractions so frequently. The reason for these discrepancies are most likely to be found in the modification of the gel technique. In the present investigation the applied gel volume was reduced compared to that used previously. This facilitates a more strict intracervical application according to the results from two recently performed studies in which 0.5 mg PGE₂ in 2.5 ml gel and 1.0 ml gel respectively was used to dilate the cervix before termination of first trimester pregnancy in young nulliparous women with a narrow cervical canal.^{3,4} In the referred studies intrauterine pressure was recorded during the gel treatment. It was then found that if the larger gel volume was applied partly extra-amniotically myometrial activity was increased. However, applied within the cervical canal the "concentrated" gel (1.0 ml) did not induce regular uterine contractions, but in spite of that a significant priming of the cervix was accomplished.^{3,4}

In nearly 70 % of our patients (Fig. 2) a favorable cervical state was found within 5 hours after the PGE₂-gel application and in these patients no further significant priming of the cervix was registered the next morning. On the other hand, in the remaining patients with still an unfavorable cervix 5 hours after the gel application a significant improvement of cervical score was registered

with unfavorable cervix

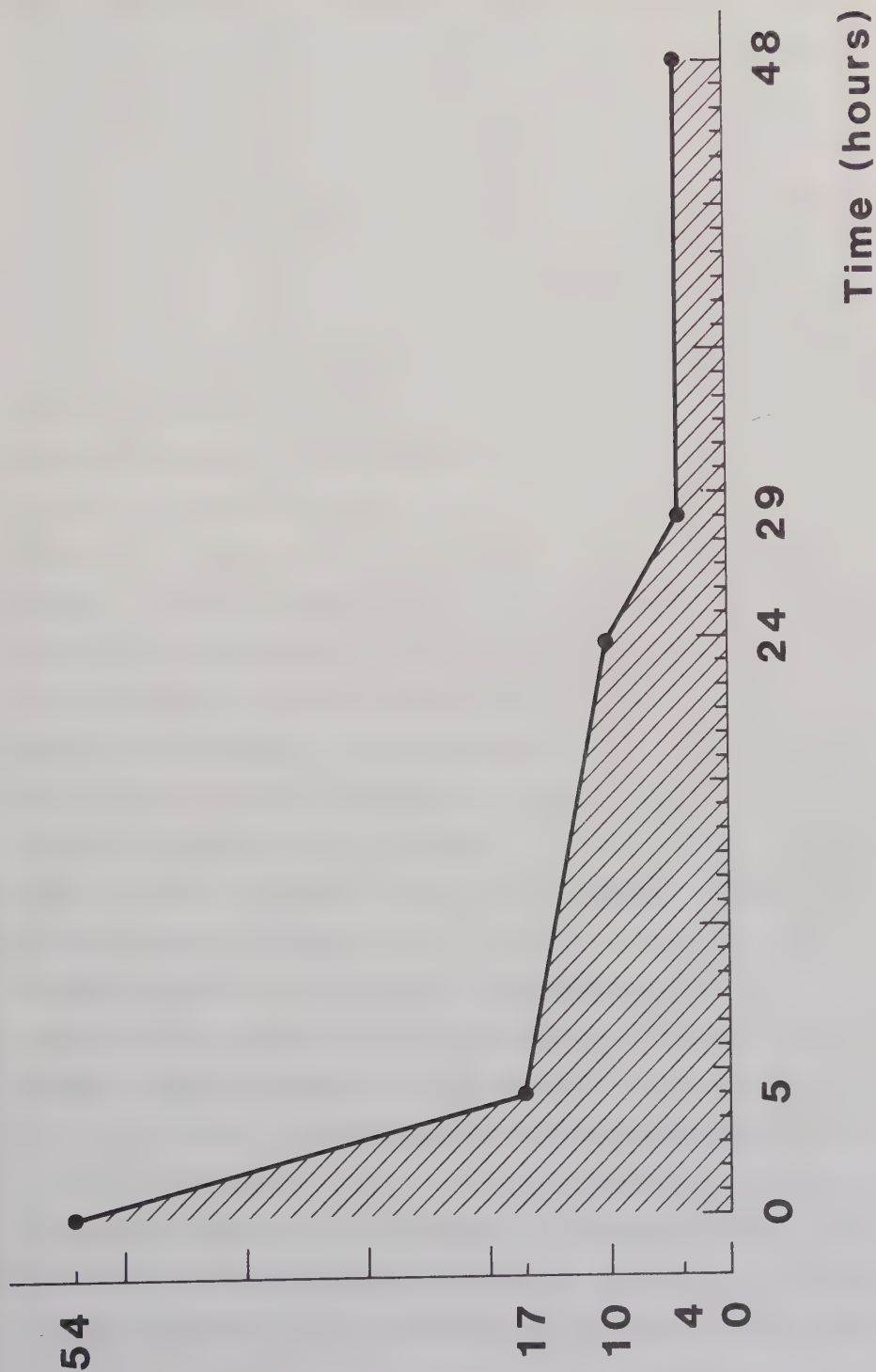


Fig. 2 Cervical ripening related to time, in fifty-four term patients with unfavorable cervix, treated with 0.5 mg PGE₂-gel intracervically.

in about half the number of women 24 hours after gel application. It can thus be concluded that in the majority of patients, a positive response to the PGE_2 -gel treatment can be expected to occur within 5 hours and in these patients no further significant improvement is likely. However, if the cervix is still unripe 5 hours after application of the PGE_2 -gel and no urgent indication of delivery is present the best policy should be to wait until the next morning since in several of these patients an improvement of cervical score will occur during the night.

Another important observation is that intravenous infusion of oxytocin in patients with a PGE_2 -gel primed cervix did not induce myometrial hypercontractility. Hence, it can be concluded that PGE_2 -gel priming of the cervix can be "combined" with the intravenous infusion of oxytocin to induce or augment labor according to the schedule used in the present study. Such a procedure may improve the induction process in patients with pregnancy complications since, if labor is induced early in the morning, delivery can be expected to occur within the same day in the majority of the patients offering optimal possibilities for intense neonatal and maternal care.

Most certainly the induction delivery time would have been reduced and number of successful deliveries within 24 hours increased if early amniotomia had been used in the

present investigation. However, this potent stimulator of labor was avoided in order to facilitate penetration of the results of the different induction procedures used in this study. In clinical routine it must, however, be emphasized that an early amniotomy will visualize meconium stained liquor amnii and provide the possibility for internal CTG, which could be most helpful to identify at an early stage a compromised fetus. Moreover, induction of labor by a combination of PGE₂-gel, amniotomy and intravenous infusion of oxytocin will most certainly increase the risk of myometrial hyperstimulation. Therefore, in our opinion, if vaginal delivery has been allowed to occur in patients with a complicated pregnancy and an unripe cervix, the first step in the induction process should be to prime the cervix. When this has been accomplished induction with amniotomy in combination with intravenous infusion of oxytocin can be instituted. Such a cautious two-step procedure facilitates the evacuation of the uterine cavity without too powerful uterine contractions compromising a delicate fetus. The total number of instrumental deliveries required in the present study (20 % including 7 % Caesarean section) must be considered low in view of the wellknown difficulties of inducing labor in patients of the category studied here and supports the previous statement. Data from our departments indicate that if induction with amniotomy and intravenous infusion of oxytocin is performed in similar patients as those presented here, one can expect an instrumental delivery

rate of approximately 30 % including 15 - 20 % Caesarean sections. These data are in accordance with those reported by Calder et al, Shepherd et al and Sims et al comparing the results of induced labor in patients after PGE₂-gel treatment with those after conventional induction with amniotomy and intravenous infusion of oxytocin.^{2,9,10}

From the present, as well as previous investigations, on different techniques to induce labor in patients with an unripe cervix, it is obvious that in some patients it seems impossible to achieve cervical priming or to induce labor. In the present study two patients out of 54, i.e. 4 %, had to be delivered by Caesarean section due to failed induction. Moreover, in another two patients repeated inductions had to be performed. The reason for the poor response in these patients is unclear and can not be answered by the present study. It can however, be discussed whether patients with an early poor response should be subjected to repeated attempts of induction. It might be better to decide to deliver these patients by Caesarean section if the second attempt to induce delivery has failed.

It is wellknown that the unripe cervix is a poor prognostic sign for induction with often prolonged labor and high incidence of fetal distress. From the results of the present study it may then be justified to conclude that intracervically applied PGE₂-gel followed by intravenous

infusion of oxytocin seems to be a safe and effective method for cervical priming and labor induction in term patients with an unripe cervix.

REFERENCES

1. Calder, A.A., Embrey, M.P. & Tait, T.: Ripening of the cervix with extraamniotic prostaglandin E_2 in viscous gel before induction of labour. *Br J Obstet Gynaecol* 84:264, 1977.
2. Calder A.A.: The management of the unripe cervix. *Human Parturition*, First edition. Edited by M Keirse, A Anderson and J Bennebroek-Gravenhorst. Leiden University Press p. 201, 1979.
3. Forman, A., Ulmsten, U. & Wingerup, L., et al: Effects of intracervical PGE_2 -gel on myometrial activity and cervical state in first trimester pregnancy. *Prostaglandins* 24:303, 1982.
4. Forman, A., Ulmsten, U., Bányai, J. et al: Evidence for a local effect of intracervical PGE_2 -gel. *Am J Obstet Gynecol*. In press.
5. Gillespie, A.: Prostaglandin-oxytocin enhancement and potentiation and their clinical applications. *Br Med J* 1:150, 1972.
6. Gillespie, A., Brummer, H.C., Chard, T.: Oxytocin release by infused prostaglandin. *Br Med J* 1:543, 1972
7. Harris, A.S., Kirstein-Pedersen, A., Stenberg, P., et al: Preparation, characterization, and stability of new prostaglandin E_2 gel for local administration. *J Pharm Sci* 69:1271, 1980.
8. Lippert, T.H.: The use of prostaglandin gel in obstetrics and gynecology. *Arch Gynecol* 227:171, 1979.
9. Sims, C.D., Mellows, H.J., Spencer, P.J., et al: Routine induction of labour with extra-amniotic prostaglandin E_2 in a viscous gel. *Br J Obstet Gynaecol* 86:529, 1979.
10. Shepherd, J.H., Pearce, J.M. & Sims, C.D.: Induction of labour using prostaglandin E_2 pessaries. *Br Med J* 2:108, 1979.
11. Thiery, M., Defoort, P., Benijts, G., et al: Effectiveness of extraovular injection of prostaglandin E_2 in Tylose^R gel to ripen the cervix prior to elective induction of labor at term. *Prostaglandins* 14:381, 1977.
12. Thiery, M.: Induction of labor with prostaglandins. *Human Parturition*. First Edition. Edited by M Keirse, A Anderson, J Bennebroek-Gravenhorst. Leiden University Press, p. 155, 1979.
13. Ulmsten, U. & Wingerup, L.: Cervical ripening induced by prostaglandin E_2 in viscous gel. *Acta Obstet Gynecol Scand Suppl* 84, 1979.
14. Ulmsten, U., Wingerup, L. & Andersson, K.-E.: Comparison of prostaglandin E_2 and intravenous oxytocin for induction of labor. *Obstet Gynecol* 54:581, 1979.
15. Ulmsten, U. & Wingerup, L.: Clinical experiences with a new gel for intracervical application of prostaglandin E_2 before therapeutic abortion or induction of term labor. *Prostaglandins* 20:533, 1980.

16. Ulmsten, U., Wingerup, L., Belfrage, P., Ekman, G. & Wiquist, N.: Intracervical application of prostaglandin gel for induction of term labor. *Obstet Gynecol* 59:336, 1982.
17. Wingerup, L., Andersson, K.-E. & Ulmsten, U.: Ripening of the uterine cervix and induction of labour at term with prostaglandin E₂ in viscous gel. *Acta Obstet Gynecol Scand* 57:403, 1978.

INTRAVAGINAL VERSUS INTRACERVICAL APPLICATION OF
PROSTAGLANDIN E_2 IN VISCOUS GEL FOR CERVICAL
PRIMING AND TERM LABOR INDUCTION IN PATIENTS
WITH AN UNFAVORABLE CERVICAL STATE

Gunvor Ekman, Axel Forman, Karel Maršál, & Ulf Ulmsten

Department of Obstetrics and Gynecology, Malmö General Hospital, Malmö and Department of Obstetrics and Gynecology, University of Aarhus, Aarhus, Denmark.

ABSTRACT

Sixty term pregnant women with unripe cervix were randomly given either 0.5 mg PGE_2 in 2 ml gel intracervically or 4 mg PGE_2 in 3 ml gel intravaginally to prime the cervix and/or to induce labor.

In patients with a highly unfavorable cervix (cervix score - 3) the intracervical application was significantly more effective than the intravaginal. In patients with a more favorable cervical state (cervix score 4 - 5) the two routes of application were equipotent. Gastrointestinal side effects were registered after intravaginal but not after intracervical application. No fetal side effects were observed and all children were born in good condition with Apgar score > 7 within 5 minutes.

Before and during gel treatment intrauterine pressure (IUP) was recorded by micro-transducers in 8 of the patients. Intracervical application of PGE_2 -gel did not increase myometrial activity significantly whereas a prominently induced activity was recorded after intravaginal gel application. In addition several patients complained of intense uterine contractions after intravaginal but not after intracervical gel application.

Local i.e. extra-amniotic, intracervical or intravaginal application of prostaglandin E_2 (PGE_2) in viscous gel has been reported an effective agent for cervical priming and/or induction of labor in term patients with an unripe cervix (1,2,3,4).

The vaginal route has gained much attraction since it implies an easy and non-invasive technique. It also offers the possibility for self administration. However, due to the relatively high doses of PGE_2 that have to be used (2 - 5 mg) and the rapid entrance of the applied compounds into systemic circulation the intravaginally applied PGE_2 -gel may be associated with negative side effects such as gastrointestinal discomfort and uterine hypercontractility (5,6,7).

Intracervical application is a somewhat more complicated procedure compared to intravaginal administration but requires on the other hand a much smaller dose of PGE_2 (0.5 mg) to induce cervical priming and/or term labor. Similarly the intracervical route seems to be associated with very few side effects (2).

The aim of the present investigation was to compare the effect of intravaginally and intracervically applied PGE_2 in viscous gel for priming of the cervix and/or induction of term labor in patients with an unfavorable cervical state.

MATERIAL AND METHODS

Sixty term pregnant women with an unripe cervix, intact membranes, head presentations and no uterine contractions participated in the study. In all patients two ultrasound scannings had been performed in the 17th and 33rd week of pregnancy to estimate a correct gestational age and to exclude placenta praevia and multiple pregnancy. Before the investigation informed consent was obtained from the subjects. The indications for induction were all obstetric like toxemia, isoimmunization, fetal growth retardation and suspected placental insufficiency.

According to parity the patients were allocated into two groups (Table I). Group A consisted of 30 nulliparous patients with a mean age of 24 years (range 19 - 41), a mean gestational age of 41 weeks (range 37 - 43), and a mean cervical score of 3.0 (range 1 - 5). In group B there were 30 parous women with a mean age of 31 years (range 20 - 43), a mean parity of 1.7 (range 1 - 3), a mean gestational age of 40.5 weeks (range 37 - 43), and a mean cervical score of 3.5 (range 1 - 5).

Randomly the patients received either 0.5 mg PGE_2 in 2 ml gel intracervically, or 4 mg PGE_2 in 3 ml gel intravaginally to prime the cervix and/or to induce labor. The intravaginal dose of PGE_2 was chosen from recently performed dose titrating studies involving 15 term patients with unripe cervix given PGE_2 in doses from 2 to 5 mg in 3

Clinical data from 60 term pregnant women with unfavorable cervix randomly given 0.5 mg PGE₂-gel intracervically (i.c.) or 4 mg PGE₂-gel intravaginally (i.v.).

	Nulliparous women (Group A)		Multiparous women (Group B)	
	(n = 30)		(n = 30)	
Mean age (years)	25 (range 19 - 41)		31.6 (range 37 - 43)	
Parity	-		1.7 (range 1 - 3)	
Gestational age (weeks)	41 (range 37 - 43)		41 (range 37 - 43)	
Mean cervical score	3.0 (range 1 - 5)		3.6 (range 1 - 5)	
	I.c. appl. of	I.v. appl. of	I.c. appl. of	I.v. appl. of
	0.5 mg PGE ₂	4 mg PGE ₂	0.5 mg PGE ₂	4 mg PGE ₂
	n = 15	n = 15	n = 15	n = 15
Mean age (years)	26.5	23.4	30.2	33
	(range 19 - 41)	(range 19 - 33)	(range 23 - 38)	(range 20 - 43)
Parity	-	-	1.6	1.7
			(range 1 - 3)	(range 1 - 3)
Mean gestational age (weeks)	41	41	40	41
	(range 38 - 48)	(range 37 - 43)	(range 38 - 43)	(range 37 - 43)
Mean cervical score	2.9	3.1	3.6	3.5
	(range 1 - 5)	(range 1 - 5)	(range 1 - 5)	(range 1 - 5)

to 5 ml gel (Ekman, Wingerup, Ulmsten, 1982 unpublished observations).

The gel was prepared as described in detail elsewhere (2). It was applied within the cervical canal using a 12 cm long and 3 mm thick nylon catheter with one side hole half a cm from the tip. The instillation was done during slowly continuous withdrawal of the catheter starting just below the internal os. Immediately after application the catheter was removed. The intravaginal gel application was done in the posterior fornix using the same type of catheter as in the intracervical application. As seen from Table I there were no significant differences between patients receiving intracervical or intravaginal gel concerning age, gestational age, parity and cervical score.

Before treatment cervical state was assessed according to a modified Bishop score (2). All patients were monitored by cardiotocography (CTG, Hewlett Packard) starting 30 minutes before gel application. For practical reasons the gel was applied early in the morning with the cervical state reassessed in all undelivered patients after 5 and 24 hours.

In all patients not induced into labor but in whom a favorable cervical state (cervical score > 5) was registered 5 hours after gel application intravenous infusion of oxytocin was instituted. Using an electronic infusion pump the oxytocin infusion started with 2 MU/minute and was subsequently increased by 2 MU/minute every half hour

up to 24 MU/minute. Amniotomy was undertaken when labor was well established and cervix dilated to ≥ 4 cm. After amniotomy or spontaneous rupture of the membranes internal cardiotocography was instituted. pH-measurements from the fetal scalp was taken whenever indicated. Hypercontractility was defined as contractions occurring more frequently than 5 per every 10 minute; a contraction amplitude exceeding 60 mmHg, and a resting tone between contractions exceeding 15 - 20 mmHg.

Patients who did not respond to the therapy, i.e. those not induced into labor and in whom cervix was still unripe 24 hours after gel application, were considered as failures and treated with another application of 0.5 mg PGE₂-gel intracervically.

In order to quantitate properly the myometrial response to the PGE₂-gel treatment intrauterine pressure (IUP) was recorded by microtransducers in 8 patients (4 after intracervical and 4 after intravaginal application). The recording technique which has been described in detail elsewhere (8,9,10) is only briefly outlined here. With the patient in lithotomy position the micro-transducer catheter was introduced into the cervical canal and placed with the recording section at the tip of the catheter extra-amniotically 3 - 4 cm above the internal os (Fig.1). Due to the flexibility and small size of the catheter this can be done relative easily by an experienced investigator

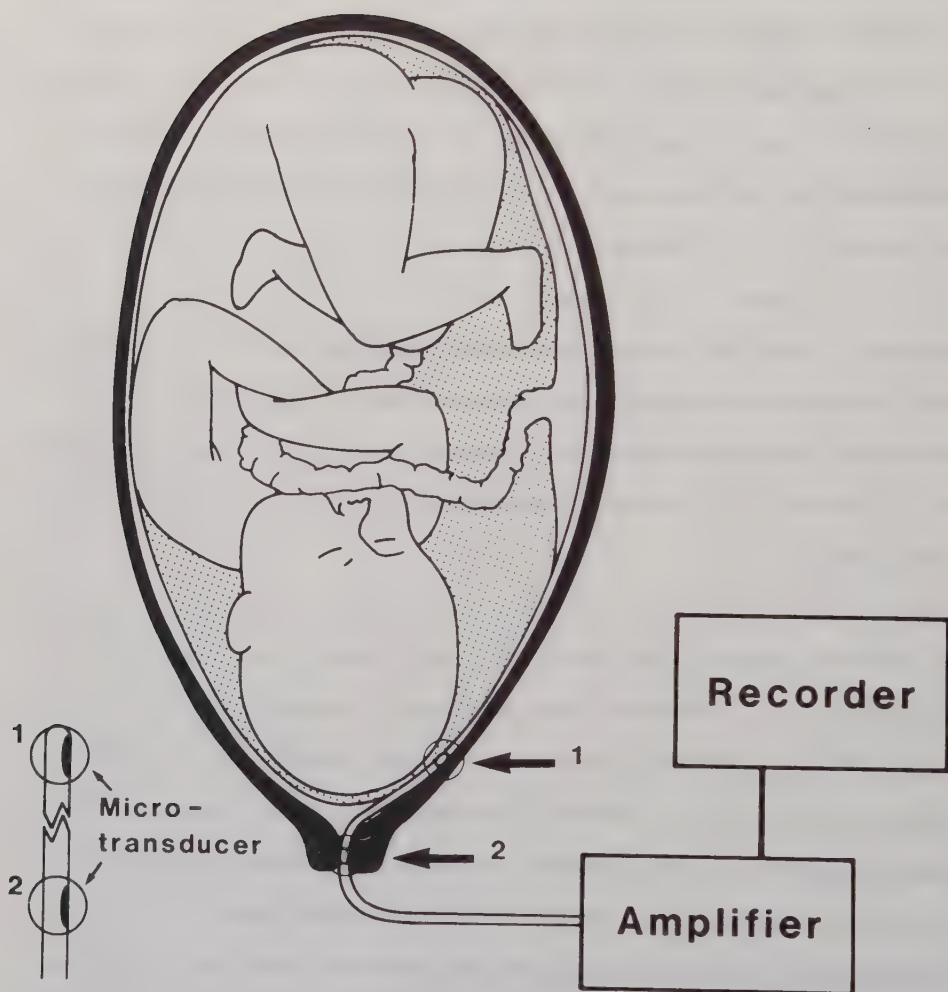


Fig. 1 The microtransducer catheter applied extra-amniotically.

Results of treatment of sixty term pregnant women with unfavorable cervix given 0.5 mg PGE₂-gel intracervically (i.c.) or 4 mg PGE₂-gel intravaginally (i.v.).

	Nulliparous women		Multiparous women	
	i.c. appl. n = 15	i.v. appl. n = 15	i.c. appl. n = 15	i.v. appl. n = 15
Favorable cervical state after 5 hours	12/15 (80%)* (cx score 3.3→6.6)	4/15 (27%) (cx score 4→8)	15/15 (100%)** (cx score 3.6→6.5)	7/15 (47%) (cx score 4.3→7.7)
Delivered within 12 hours	10/15 (67%)*	4/15 (27%)	12/15 (80%)	8/15 (53%)

* p < 0.05

** p < 0.01

According to Yates' test

without rupturing of the membranes. The catheter was fixed in position by adapters to the patients thigh. The patient was then allowed to move freely in bed. The pressure signals obtained were amplified and registered on a pen recorder (Gould, USA). Gel application was not performed until possible myometrial activity had been characterized by the IUP recordings for about one hour. The registration was then continued for another 4 hours whereafter the catheter was removed and cervical score reassessed.

RESULTS

The results are summarized in Tables II, III, Fig. 2, 3, 4, and 5. In group A, 12 out of 15 nulliparous patients had a favorable cervical state 5 hours after intracervical gel application (improvement of cervical score from mean 3.3 to mean 6.6) and 9 were delivered within 12 hours (Table II). Only one patient in this group still had an unripe cervix 24 hours after application of the gel. Among the 15 nulliparous women who received 4 mg PGE₂-gel intravaginally 4 out of the 15 had a favorable cervical state after 5 hours (cervical score improved from mean 4 to mean 8) and all were delivered within 12 hours. Four women of the 15 still had an unripe cervix 24 hours after application of the gel. The difference in cervical priming after intracervical and intravaginal gel treatment respectively is statistically significant ($p < 0.05$) as is the

% Favourable cervix 5 hours after application

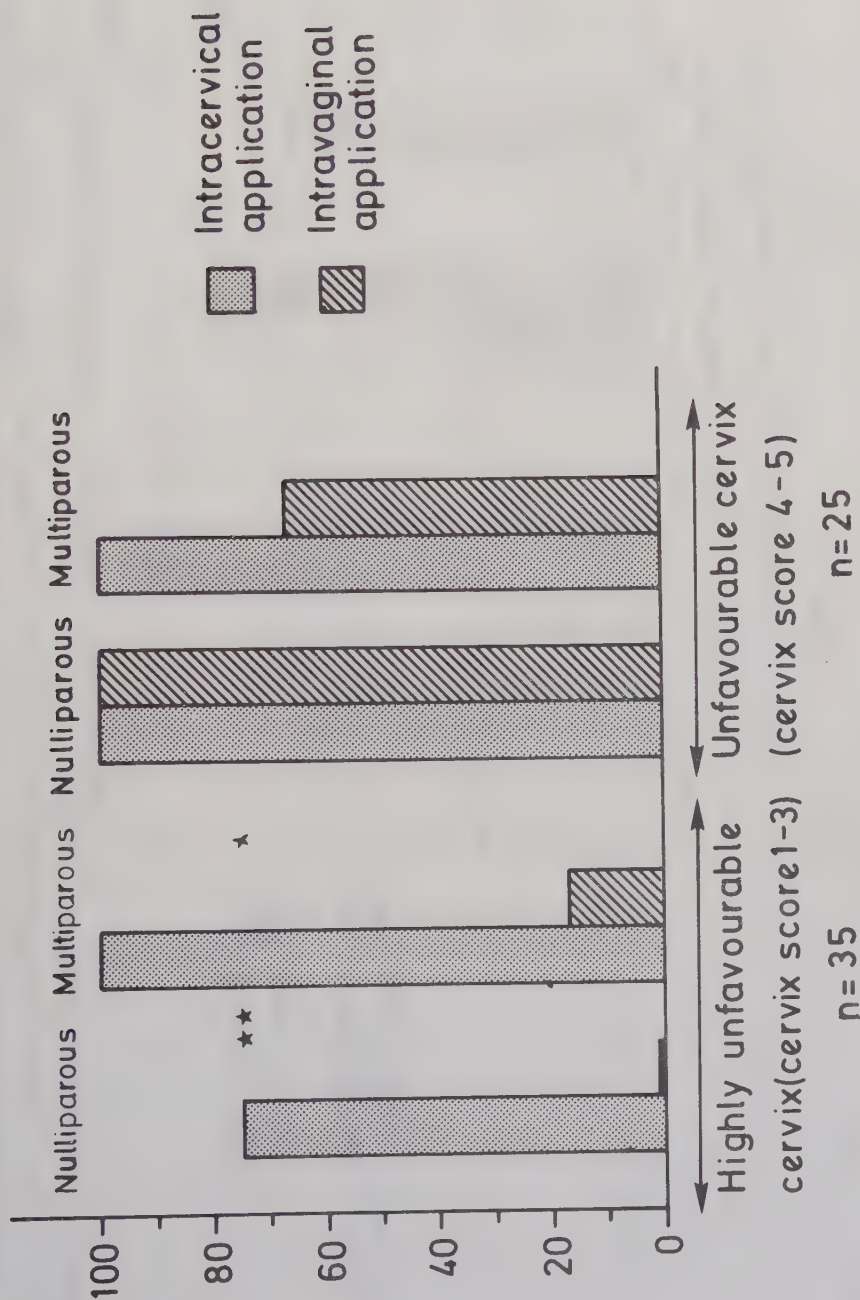


Fig. 2 The number of women (in per cent) with favorable cervix 5 hours after intracervical application of 0.5 mg PGE₂ or intravaginal application of 4 mg PGE₂ in viscous gel. The patients are allocated to four groups according to parity and original cervical state.

% delivered <12 hours after application of the gel

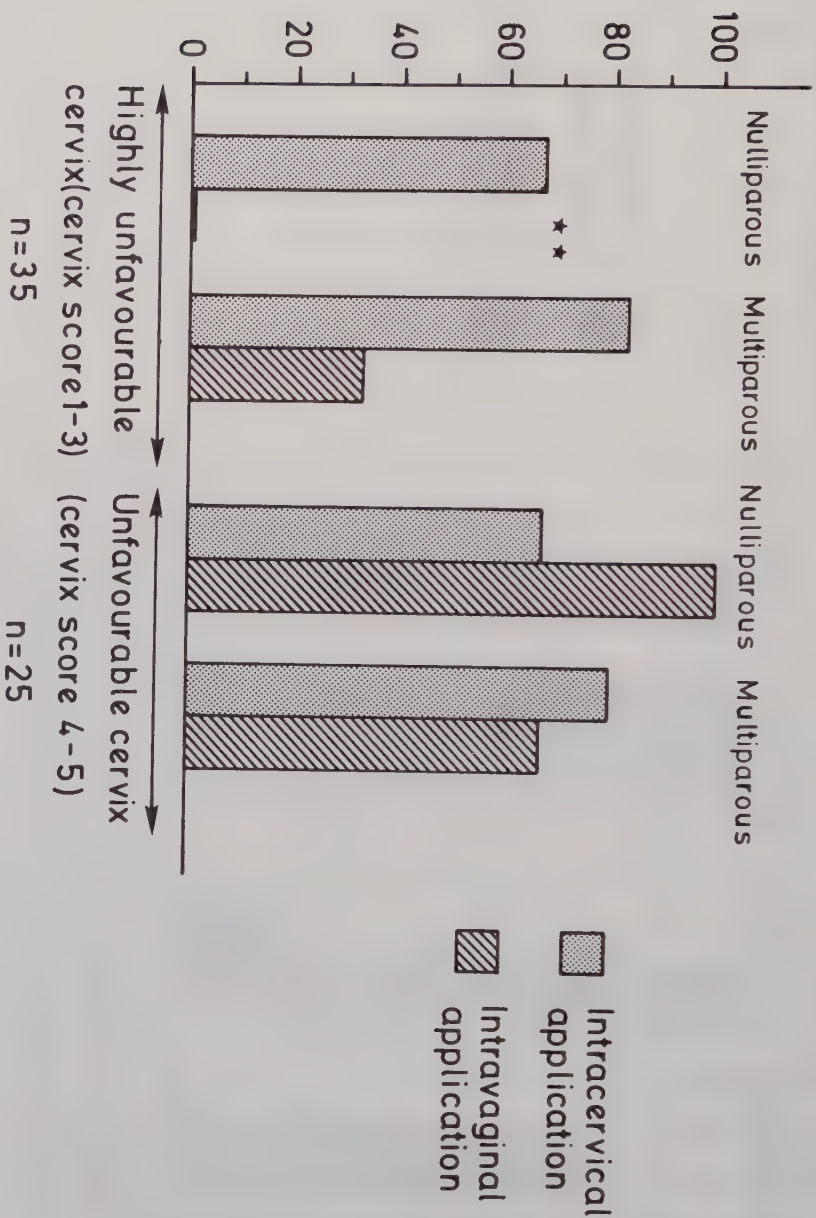


Fig. 3 The number of women (in per cent) delivered within 12 hours after intracervical application of 0.5 mg PGE₂ or intravaginal application of 4 mg PGE₂ in viscous gel. The patients are allocated to four groups according to parity and original cervical state.

Results of treatment of thirty-five patients with highly unfavorable cervix (cervixscore ≤ 3), given 0.5 mg PGE₂-gel intracervically (i.c.) or 4 mg PGE₂-gel intravaginally (i.v.). Upper panel includes the whole material. Lower panel presents cervical priming according to parity.

Women with highly unfavorable cervix
n = 35

i.c. appl. i.v. appl.
n = 18 n = 17

15/18 (83%)*
1/17 (6%)

Favorable cervical state
after 5 hours

Nulliparous women Multiparous women
n = 23 n = 12

i.c. appl. i.v. appl. i.c. appl. i.v. appl.
9/12 (75%)*
0/11 (0%) 6/6 (100%)* 1/6 (17%)

Favorable cervical state
after 5 hours

* p < 0.05

** p < 0.01

*** p < 0.001

According to Yates' test

difference in the number of delivered patients ($p < 0.05$) (Table II).

In group B all the 15 multiparous patients given intra-cervical gel had a favorable cervical state 5 hours after gel application and 12 were delivered within 12 hours. In contrast, only 7 of the 15 patients had a favorable cervical state after 5 hours and were delivered within 12 hours after vaginal gel application. These differences are statistically significant ($p < 0.01$) (Table II). Three patients still had an unripe cervix 24 hours after intra-vaginal gel application compared to none after intra-cervical application.

If one analyzes the outcome in patients with a highly unfavorable cervix, i.e. an initial cervical score of ≤ 3 the differences between the two modes of treatment are highly significant $p < 0.001$ (Table III, Fig. 2, 3). However, in patients with a "moderately unfavorable" cervix, i.e. a cervical score 4 - 5, the differences are smaller. They are in fact not significant (Fig. 2, 3).

The results of the IUP-recordings are summarized in Fig. 4 and 5. As is clearly seen from Fig. 4, which illustrates typical recordings from two patients, introduction of the recording catheter did not induce any significantly increased myometrial activity. Intracervical application of PGE_2 -gel affected the myometrial activity but little,

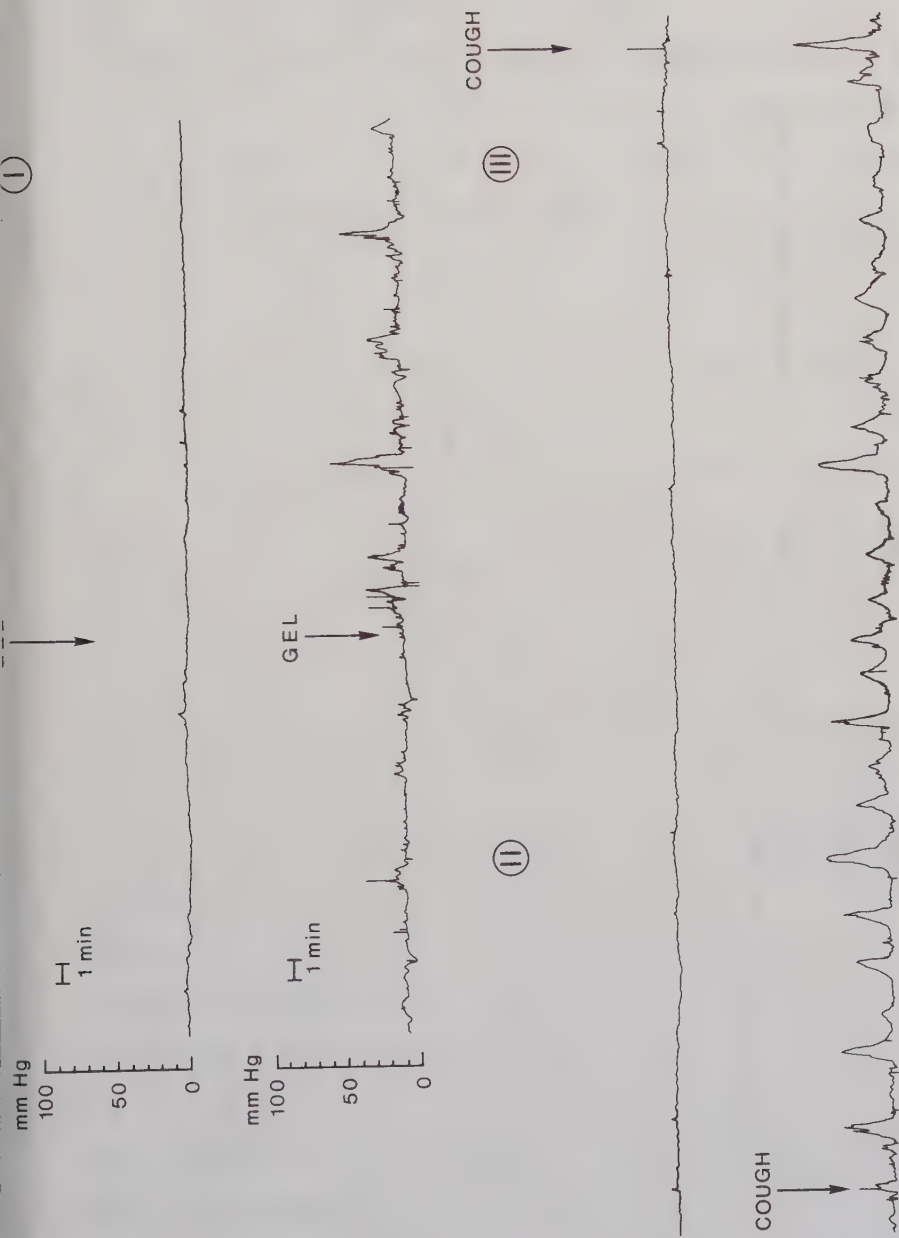


Fig. 4 The myometrial activity registered by an extraamniotically applied microtransducer catheter 30 (I), 60 (II), and 180 (III) minutes after PGE₂ gel application. Upper tracing: Intracervical application of 0.5 mg PGE₂. Lower tracing: Intravaginal application of 4 mg PGE₂.

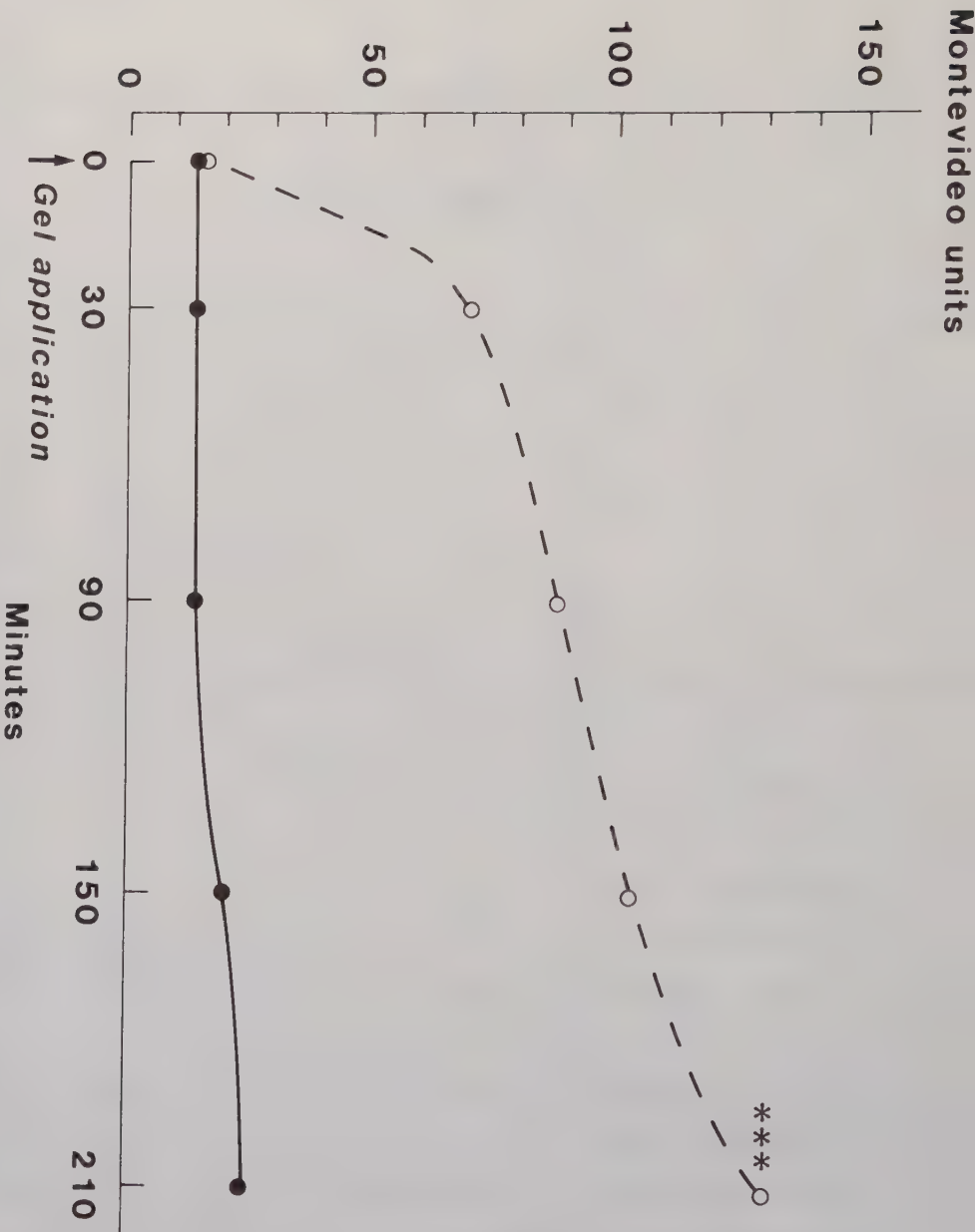


Fig. 5 The myometrial activity expressed in Montevideo units after intracervical application of 4 mg PGF₂ gel (—) or intravaginal application of 0.5 mg PGF₂ gel (---).

whereas in contrast prominent uterine contractions were seen within 30 minutes after intravaginal application. As seen from Fig. 5 there was significant difference in myometrial activity as expressed in Montevideo units after intravaginal gel application compared to intracervical. The improvement in cervical score after 5 hours did not differ significantly between the two groups of recorded patients.

No maternal side effects were noticed after intracervical application. However, among patients receiving intravaginal gel three women had episodes of violent vomiting. Moreover, unlike those who received intracervical gel, several patients complained of painful uterine contractions after intravaginal gel application. In 5 of these patients powerful uterine contractions were registered and three women had to receive analgetics during cervical priming. According to our strict definitions, the recorded myometrial activity could, however, not be classified as hyperstimulation. One patient had to be delivered by Caesarean section after intracervical application and four after intravaginal. The indication for Caesarean section was in all patients signs of fetal distress.

The eight patients (one after intracervical and seven after intravaginal treatment) in whom cervical priming and/or induction failed, i.e. labor was not induced and cervix was still unripe 24 hours after gel application,

received another intracervical gel application and were delivered within the next day.

Irrespective of the mode of treatment and delivery all children were born in good condition with Apgar score > 7 within 5 minutes. In patients subjected to IUP recordings the introduction of the catheter did not cause any discomfort. Thus no bleeding or rupturing of the membranes occurred and no signs of infection attributed to the recording procedure were registered post partum.

DISCUSSION

To our knowledge no prospective comparative studies on intracervical and intravaginal gel application for cervical priming and/or induction of labor have been carried out. The results from the present investigation indicate that in patients with a relatively ripen cervix there are small differences in efficacy between the two routes of application. However, especially in patients with a highly unfavorable cervix the intracervical application is significantly more effective than the intravaginal.

Most important when discussing different therapies for term labor induction are, however, the potential maternal and fetal side effects. In the present investigation like in many previous studies no serious adverse effects were registered after intracervical application of 0.5 mg PGE₂

in gel (12,13). After intravaginal application of 4 mg PGE₂ three patients had evident signs of gastrointestinal discomfort. Moreover, several patients complained of intense uterine contractions and three women required analgetics. That intravaginal PGE₂-gel administration induces a considerable myometrial activity compared to the intracervical was objectively confirmed by the IUP recordings. The reason for the more pronounced side effects after intravaginal application is most certainly the prompt absorption of the prostaglandin compound into systemic circulation as pointed out previously by Gordon-Wright and Elder (7). Most probably the related side effects can be reduced if the dose of intravaginally applied PGE₂ is reduced. According to the results from studies with different doses of PGE₂-gel this would, however, reduce the effectiveness of the treatment (Ekman, Wingerup, Ulmsten unpublished observation 1982).

The present investigation did not include any placebo treatment. From several previous reports on intravaginal and intracervical PGE₂-gel application, double blind studies have, however, shown that it is the prostaglandin compound and not the gel vehicle or its application that induces cervical priming and labor (12,13,14,15). Moreover, previous studies using the present IUP recording technique have shown that application of the recording catheter and a placebo gel does not increase myometrial

activity significantly and does not bring about cervical priming (16,17).

In the present study no serious maternal or fetal side effects were registered. Moreover, the number of Caesarean sections 5 out of 60 patients, i.e. 8 % must be considered low in view of the category of patients studied. Taken together previous and present investigations suggest that local application of PGE_2 in viscous gel can be used for cervical priming and/or labor induction in term patients with an unfavorable cervical state. In patients with a highly unfavorable cervix intracervical application seems to be superior to intravaginal both concerning cervical priming and induction of labor. In patients with a more favorable cervix (cervical score 4 - 5) the two routes of gel application seem to be equipotent. Side effects are more likely to occur after intravaginal application.

REFERENCES

1. MacKenzie, I.Z.: Clinical studies on cervical ripening. In: The cervix in pregnancy and labor. Clinical and biochemical investigations. Ed. D.A. Ellwood, A.B.M. Anderson. Churchill Livingstone 163, 1981.
2. Ulmsten, U. & Wingerup, L.: Acta Obstet Gynecol Scand, Suppl 84, 1979.
3. Calder, A.A.: The management of the unripe cervix. In: Human Parturition. Keirse M. JNC, Anderson A.B.M., Bennebroek-Gravenhorst J. (ed). Leiden University Press. 201, 1979.
4. Shepherd, J.H. & Knuppel, R.A.: Role of prostaglandins in ripening the cervix and inducing labor. Clin in Perinatol 8:49, 1981.
5. Davey, D.A., Domisse, J. & Mac Nab, M.: Intravaginal prostaglandin E₂ for cervical ripening and induction of labour. South African Medical Journal 58:516, 1980.
6. MacKenzie, I.Z. & Embrey, M.P.: Cervical ripening with intravaginal prostaglandin E₂ gel. British Medical Journal 2:1381, 1977.
7. Gordon-Wright, A.P. & Elder, M.G.: The systemic absorption from the vagina of prostaglandin E₂ administered for the induction of labor. Prostaglandins 18:153, 1979.
8. Lindström, K. & Ulmsten, U.: Some methodological aspects on the measurement of intraluminal pressures in the female urogenital tract in vivo. Acta Obstet Gynecol Scand 57:63, 1978.
9. Ulmsten, U. & Andersson, K.-E.: Multi-channel intra-uterine pressure recording by means of micro-transducers. Acta Obstet Gynecol Scand 58:115, 1979.
10. Ulmsten, U., Andersson, K.-E., Lindström, K. & Persson, P.H.: Determination of myometrial tension during labor by combined micro-transducer IUP-recording, and ultrasonic examination of uterine cavity. Acta Obstet Gynecol Scand 58:547, 1979.
11. Gordon-Wright, A.P. & Elder, M.G.: Prostaglandin E₂ tablets used intravaginally for the induction of labour. Brit J Obstet Gynaecol 86:32, 1979.
12. Wingerup, L., Andersson, K.-E. & Ulmsten, U.: Ripening of the uterine cervix and induction of labor at term with prostaglandin E₂ in viscous gel. Acta Obstet Gynecol Scand 57:403, 1978.
13. Ulmsten, U., Wingerup, L., Belfrage, P., Ekman, G. & Wiqvist, N.: Intracervical application of prostaglandin gel for induction of term labor. Obstet Gynecol 59:336, 1982.
14. Calder, A.A., Embrey, M.P. & Tait, T.: Ripening of the cervix with the extraamniotic prostaglandin E₂ in viscous gel before induction of labour. Br J Obstet Gynaecol 84:264, 1977.

15. MacKenzie, I.Z. & Embrey, M.P.: A comparison of PGE₂ and PGF_{2α} vaginal gel for ripening the cervix before induction of labour. Br J Obstet Gynaecol 86:167, 1979.
16. Forman, A., Ulmsten, U., Wingerup, L. & Bányai, J.: Effects of intracervical PGE₂-gel on myometrial activity and cervical state in first trimester pregnancy. Prostaglandins 24:303, 1982.
17. Forman, A., Ulmsten, U., Bányai, J., Wingerup, L. & Uldbjerg, N.: Evidence for local effect of intracervical PGE₂-gel. Am J Obstet Gynecol 1982. In press.

RIPENING OF THE HUMAN UTERINE CERVIX RELATED
TO CHANGES IN COLLAGEN GLYCOSAMINOGLYCANS
AND COLLAGENOLYTIC ACTIVITY

Niels Uldbjerg, Gunvor Ekman, Anders Malmström,
Kjell Olsson, Ulf Ulmsten

Departments of Physiological Chemistry, University of Lund, Department of Obstetrics and Gynecology and Clinical Chemistry, Malmö General Hospital, University of Lund, Sweden and Institute of Pharmacology, University of Århus, Århus, Denmark.

ABSTRACT

Connective tissue in biopsies taken from the lower part of the uterine cervix in 40 pregnant women at various gestational ages was compared to that in similar biopsies from 15 non-pregnant women.

The concentration of collagen, sulphated glycosaminoglycans and hyaluronic acid decreased during pregnancy. At the gestational age of 10 weeks' the collagen concentration was 70 % and at term 30 % of that in the non-pregnant cervix. After delivery no further decrease was observed. The extractability of collagen increased during pregnancy as well as during labor. Also the water concentration increased.

An increase in the collagenolytic activity was observed by advancing gestational age. A DNP-peptide hydrolytic activity (collagenase) and the concentration of leucocyte elastase increased gradually by a factor of 10.

The physiological importance of the collagen was also demonstrated, since the cervical dilatation time during spontaneous labor was long in women with high collagen concentrations and short in women with low collagen concentrations.

The softening, dilatation and effacement of the human uterine cervix during pregnancy and labor does not occur as a result of uterine contractions only but is also due to an active ripening process within the cervix (1, 2, 3). Abnormalities herein may cause considerable obstetric problems compromising the child as well as the mother. Extensive ripening before term may lead to abortion or preterm delivery, whereas absence of normal ripening at term is a bad prognostic sign indicating prolonged labor or postterm delivery (4).

The human uterine cervix is dominated by a fibrous connective tissue with great amounts of collagen fibers separated by ground substance (5). Seventy per cent of the collagen is type I and thirty per cent type III (6, 7). Its degradation is determined by the two enzymes collagenase and elastase. The human cervix contains small dermatan sulphate proteoglycans (90 kDa) (8) which are supposed to stabilize the collagen (9). Also heparan sulphate is present (10), most probably it is localized to the walls of the blood vessels.

Earlier data concerning biochemical changes in human cervical connective tissue are not consistent and deal mostly with cervical biopsies from non-pregnant and post partial patients (5).

In the present investigation we have followed the concentrations of water, collagen, glycosaminoglycans as well as the collagenolytic activity in the human uterine cervix during pregnancy and parturition in order to elucidate if changes herein could explain the cervical ripening.

MATERIAL AND METHODS

After informed consent 55 women were allocated into five groups. Biopsies from the lower part of the cervix were obtained.

Non-pregnant

Group A. Ten menstruating women with a mean age of 49 years (range 36 - 52) and a mean parity of 1.5 (range 0 - 3) admitted for hysterectomy due to benign myomas of the corpus uteri. Stromatic tissue from the distal portion of the cervix was taken immediately after hysterectomy and epithelium and mucus filled crypts were carefully avoided during the sampling procedure.

Group B. Five menstruating women with a mean age of 43 years (range 33 - 49) and a mean parity of 1.7 (range 0 - 3) admitted for benign diseases not involving the cervix. The cervical biopsies were taken vaginally by a rotating needle with a diameter of 3 mm penetrating to a depth of 12 mm. The epithelium was cut away, where upon the sample weighed 10 - 30 mg.

Early pregnancy

Group C. Twenty-two nulliparous women with a mean age of 24 years (range 23 - 28) and a mean gestational age of 11 weeks (range 10 - 12 weeks) admitted for legal abortion. Biopsies were taken vaginally in the same manner as in group B.

Term pregnancy

Group D. Seven women with a mean age of 29.4 years (range 20 - 36), a mean parity of 0.85 (range 0 - 2) and a mean gestational age of 39 weeks (range 38 - 40) subjected to elective caesarean section due to suspected fetopelvic disproportion. The mean cervical score according to a modified Bishop score was 5 (range 4 - 7) (11). The biopsies were taken transvaginally by scissors and tweezers. The weight of the biopsies varied between 50 and 75 mg.

After delivery

Group E. Eleven women with a mean age of 26.5 years (range 18 - 36), a mean parity of 0.1 years (range 0 - 1) and a mean gestational age of 40 weeks (range 37 - 42) had normal spontaneous deliveries. Cervical biopsies were obtained by scissors and tweezers immediately after delivery. The weight of the biopsies varied between 50 and 100 mg. All biopsies were immersed into liquid nitrogen within 3 minutes after sampling and then stored at -60°C .

Water and glycosaminoglycans

The water content of the biopsies was defined as the difference between the wet weight and the dry weight. The latter was determined after heating the sample at 110°C in vacuum over P_2O_5 for at least five hours, after which a constant weight was obtained. The dry sample was delipidated with acetone and digested with papain. The digest was applicated at a column with 1 ml DEAE cellulose in acetate form. Hyaluronic acid was eluted with 1.1 M pyridine acetate pH 5 and sulphated glycosaminoglycans with 3.0 M pyridine acetate pH 5, and lyophilized. The hyaluronic acid containing fraction was subjected to alkaline degradation according to Carlsson (12) and applicated to a column containing 0.5 ml DEAE cellulose. The oligosaccharides from mucin glycopeptides were eluted with 0.4 M pyridine acetate and the purified hyaluronic acid with 1.2 M pyridine acetate. Sulphated glycosaminoglycans and hyaluronic acid were hydrolyzed in 6 M HCl, 100°C for eight hours, lyophilized and quantitated as hexosamine (13).

Collagen concentration and extractability: The biopsies were cut into pieces smaller than 2 mm and delipidated with acetone. They were initially extracted three times with 1 ml 0.5 M acetic acid and then three times with 1 ml 0.5 M acetic acid containing pepsin (1 mg/ml) (7). Extrac-

tions (23 hours) and centrifugations (one hour at 20,000 x g) between these were performed at 4°C. The material was hydrolyzed in 2 ml 6 M HCl at 100°C for 17 hours, lyophilized and quantitated as hydroxyproline according to Stegeman (14).

Collagenolytic activity: Extractions were performed essentially as described by Kitamura (15). The biopsy was crushed by 10 tons pressure during cooling with liquid nitrogen and then homogenized in 13 volumes of 0.25 % Triton X-100, 50 mM Tris pH 7.3 containing 100 mM CaCl₂ in a TRI-R Tissue homogenizer, S61 for four minutes at 3,000 rpm. The homogenate was centrifuged at 15,000 x g for 20 minutes at 2°C, whereafter the supernatant was diluted with extraction buffer. The hydrolytic activity against DNP-peptide was then assayed as described by Masui et al. (16). The incubation time was 2 hours and the extraction buffer was used as blank.

Leucocyte elastase was determined in the same extractions with a RIA-method (17).

Chemical reagents

Papain (twice crystallized, 16 - 40 BAAE units/mg) were purchased from Sigma Chemical Co., U.S.A., DEAE cellulose ion exchanger (DE-52) from Whatman Ltd., England, and 2,4 dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg (DNP-peptide) from the Protein Research Foundation, Japan. All other chemicals were of analytical grade.

RESULTS

The sampling procedure did not cause any complications. The rotating needle procedure facilitated sampling in the non-pregnant patients as well as in patients in early pregnancy.

The water concentration in the cervix was increased in late pregnancy (85.9 %) and post partum (87.3 %) compared to the non-pregnant state (80.8 %), $p < 0.001$. (Table I).

The hydroxyproline concentration (collagen) in samples taken at hysterectomy (group A) was $21.2 \pm 1.6 \mu\text{g/mg}$ wet weight. That is the same as in needle biopsies from non-pregnant women (Table I). During pregnancy a gradual decrease was found (Table I, and Fig. 1). Also the physiochemical properties of the collagen changed. Thus the extractability in acetic acid with pepsin was much higher during pregnancy and labor compared to the non-pregnant state (Table I). The extractability in acetic acid was, however, unchanged.

The concentration of sulphated glycosaminoglycans and hyaluronic acid was significantly lower in samples taken after delivery compared to those obtained from non-pregnant women (Table I). When the concentrations of sulphated glycosaminoglycans and hyaluronic acid were calculated as

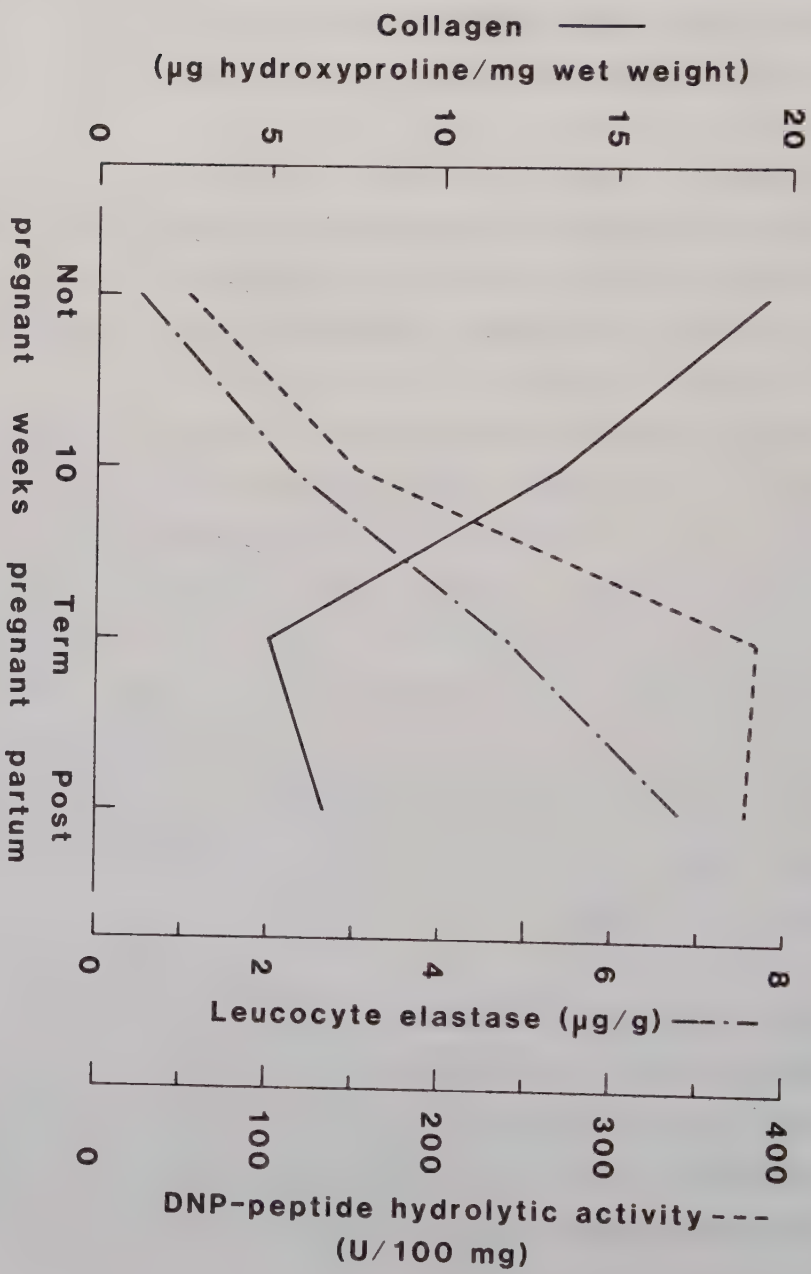


Fig. 1 Hydroxyproline (collagen), DNP-peptide hydrolytic activity and leucocyte elastase concentration in the lower part of the human uterine cervix in non-pregnant women of fertilage; in pregnant women and immediately post partum.

μ g-hexosamine per mg dry weight instead of per mg wet weight the p values were found to $p < 0.02$ and < 0.05 respectively.

In patients with spontaneous effective uterine contractions (group E) a good correlation was found between the course of delivery measured as cervical dilatation and total collagen (hydroxyproline) concentration in the cervix (Fig. 2).

No correlation was found between cervical dilatation time and DNP-peptide hydrolytic activity, elastase concentration or water concentration.

The collagenolytic activity measured as DNP-peptide hydrolytic activity and leucocyte elastase concentration was markedly increased during pregnancy congruent to the fall in the concentration of collagen (Fig. 1). 60 - 100 % of the DNP-peptide hydrolytic activity was inhibited by EDTA, 12.5 mM, 0 - 20 % by diisopropylphluorophosphate, 5 mM and 0 % by N-ethymalenimide, 5 mM. As seen from Table I the concentration of hydroxyproline and the DNP-peptide hydrolytic activity were the same at term and after delivery, whereas the concentration of leucocyte elastase was further increased post partum ($p < 0.05$, single sided t-test).

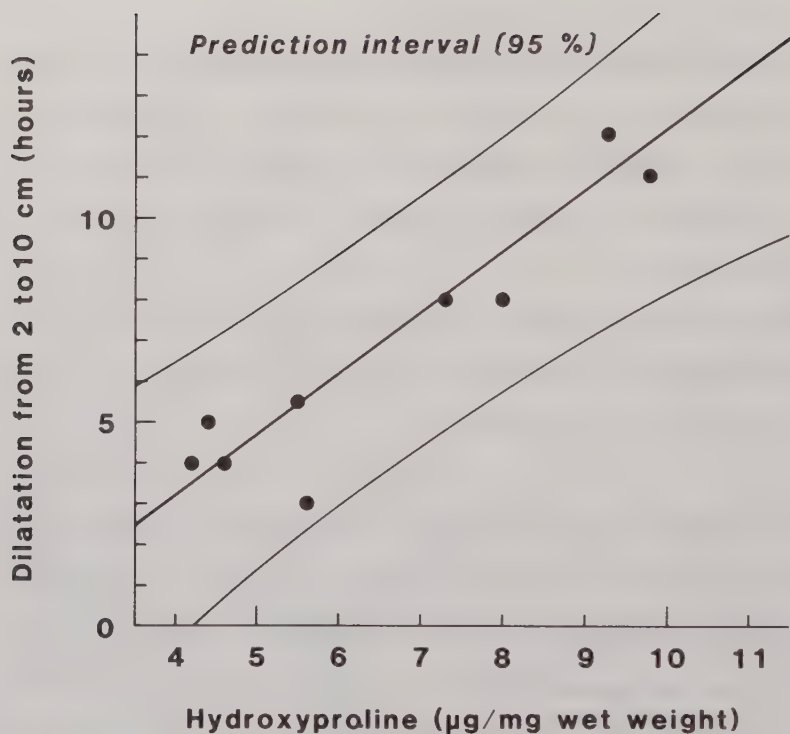


Fig. 2 Cervical dilatation time as a function of hydroxyproline concentration. The hydroxyproline concentration was determined in cervical biopsies taken immediately after delivery. The dilatation-time was defined as the time used for the cervix to dilate from 2 cm to 10 cm during established labor. The best least-squares regression line is given as well as the 95 % prediction-interval (assuming t-distribution). The correlation coefficient was 0.93 and the coefficient of determination $0.93^2 = 0.87$.

Connective tissue components and collagenolytic activity in biopsies from the human uterine cervix during pregnancy and labor. All values are given as mean $\pm$ s.e.m.. No; states the number of patients. Significantly different from non-pregnant control (single sided t-test):*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

	Non-pregnant	10 weeks' pregnancy	Late pregnancy	Post partum
Hydroxyproline:	(No 5)	(No 3)	(No 6)	(No 11)
Total ($\mu\text{g}/\text{mg}$ wet weight)	19.5 $\pm$ 1.8	13 $\pm$ 1.4*	4.95 $\pm$ 1.4***	6.7 $\pm$ 0.6***
Extractability in acetic acid (%)	0.7 $\pm$ 0.2	1.5 $\pm$ 0.2*	1.2 $\pm$ 0.3	0.9 $\pm$ 0.1
Extractability in pepsin (%)	17.0 $\pm$ 2.5	75.0 $\pm$ 8.2***	76.8 $\pm$ 3.2***	87.7 $\pm$ 1.4***
Not extractable (%)	82.3 $\pm$ 2.7	23.3 $\pm$ 8.4***	21.1 $\pm$ 3.7***	11.3 $\pm$ 1.4***
Water (% of wet weight)	(No 7) 80.8 $\pm$ 0.8	(No 10) 80.4 $\pm$ 0.7	(No 5) 85.9 $\pm$ 0.5***	(No 3) 87.3 $\pm$ 0.5***
Sulphated glycosaminoglycans (μg hexosamine/ mg wet weight)	(No 7) 0.30 $\pm$ 0.02	(No 9) 0.32 $\pm$ 0.02	(No 4) 0.17 $\pm$ 0.01**	(No 3) 0.16 $\pm$ 0.03**
Hyaluronic acid (μg hexosamine/ mg wet weight)	(No 7) 0.11 $\pm$ 0.01	(No 4) 0.09 $\pm$ 0.01	(No 4) 0.08 $\pm$ 0.005*	(No 4) 0.06 $\pm$ 0.02**
DNP-peptide hydrolytic activity (U/100 mg wet weight)	(No 10) 53.9 $\pm$ 10.7	(No 6) 150 $\pm$ 17***	(No 7) 388 $\pm$ 59***	(No 11) 380 $\pm$ 26***
Leucocyte elastase ($\mu\text{g}/\text{g}$ wet weight)	(No 7) 0.51 $\pm$ 0.06	(No 3) 1.43 $\pm$ 0.12***	(No 7) 4.82 $\pm$ 1.03***	(No 11) 6.81 $\pm$ 0.44***

DISCUSSION

The human uterine cervix is a "heterogenous" organ containing fibrous connective tissue, smooth muscle, blood vessels, epithelium and mucus filled crypts extending deep into the stromatic tissue. The distribution of these components varies within the organ. Thus, the lower part of the cervix contains more connective tissue and the upper part relatively more smooth muscle (18). It is therefore important to know from which part of the organ the specimens have been taken. The biopsies used in this study are most probably representative for the stromatic tissue in the lower cervix since collagen concentration and DNP-peptide hydrolytic activity was the same in biopsies taken vaginally and in stroma sample obtained at hysterectomy.

Hydroxyproline determination is an accepted method for collagen quantitation, although this amino acid is also found in a few other but quantitatively less important molecules (19). Assuming 12 % hydroxyproline in the collagen, the latter constitutes not less than 85 % of the dry material in the non-pregnant human cervix (Table I). The major part is not extractable suggesting it to be relatively old collagen with several and acid stable cross-links.

The decrease in collagen concentration found already early in pregnancy is in accordance with the observed clinical softening of the cervix at this stage. Moreover, the increased extractability indicates the old not-extractable collagen to be broken down and replaced by extractable collagen. This remodulation of the cervix is still more marked at term (Fig. 1 and Table I). It is worthy to note that no significant change in collagen concentration was observed after delivery. Most likely the remodulation of the spontaneously ripened cervix is more or less completed at the onset of labor. Another possibility as suggested by Kleissl et al. (6) is that partly degraded collagen is retained within the tissue for some time. If so, this could explain why the collagen concentration was unchanged in spite of a high activity of collagenolytic enzymes (Fig. 1) and why the extractability increases during labor (Table I).

Since the collagen concentration did not change during labor the relationship between cervical collagen concentration and cervical dilatation time as shown in Fig. 2 should be the same if the biopsies had been taken before start of labor. Hence the collagen concentration in the cervix before onset of labor could be used to predict the course of the delivery in terms of cervical dilatation time. As seen from Fig. 2 the coefficient of determination is 85 %. Assuming an independence between cervical collagen concentration and uterine contractions this means that interindividual differences in the efficacy of labor

determines less than 15 % of the variation in dilatation time. It is, however, most likely that the collagen degradation in the cervix and the "condition" i.e. state of excitability of the myometrium to some extent are regulated by common factors, e.g. the local hormonal milieu. Accordingly patients with little myometrial activity or insufficient uterine contractions should be those with low cervical compliance i.e. high concentration of cervical collagen. Determination of the concentration of hydroxyproline may therefore not only describe the ripeness of the cervix and predict its ability to dilate but also suggest the "contractile potential" of the myometrium.

The decrease in concentration of sulphated glycosaminoglycans was less than the decrease in collagen concentration. This is probably due to an increase in heparan sulphate opposing the decrease in dermatan sulphate (10). The increase in heparan sulphate concentration may be due to an increased vascularisation of the organ. It is obvious that previous histological descriptions of an increased amount of amorphous material between the collagen fibers post partum are not due to dermatan sulphate proteoglycans (5).

The present data suggest the decrease in collagen concentration to be associated with the DNP-peptide hydrolytic activity and the concentration of leucocyte elastase. There are good reasons to believe that the DNP-peptide is

reasonable specific to collagenase (16). This enzyme is present in both active and inactive forms; the active form constitutes only 15 % of total collagenase in extracts from the human uterine cervix (15,20). The major part of the inactive collagenase is bound to a α_2 -macroglobulin. It is not known whether this binding is formed in vivo or whether it is an artefact produced during the extraction procedure. Since the DNP-peptide hydrolytic activity expresses the presence of both active collagenase and collagenase bound to α_2 -macroglobulin, it is a good substrate to use for collagenase determination if the enzyme inhibition is an artefact. If it is formed in vivo the complex may be considered important for the regulation of the collagenase activity.

The presence of leucocyte elastase indicates a role of leucocytes in the ripening process as suggested by Junqueira et al. who found a dissolution of the connective tissue around leucocytes in the uterine cervix after delivery (21).

In the present study we have only investigated some of the important constituents of the human uterine cervix. Thus, we have e.g. not penetrated the importance of elastin (22) or contractile elements on the state of cervix (23). Based on the present findings it may, however, be justified to conclude that cervical ripening as clinically characterized by softening, effacement and dilatation is accompa-

nied with prominent changes in the composition of collagen and glycosaminoglycans in the cervical connective tissue.

ACKNOWLEDGEMENT: The research was supported by grants from the Danish Medical Research Council (J.Nos 12-2264), from the Swedish Medical Research Council (5670), the Medical Faculty, University of Århus and the Medical Faculty, University of Lund.

REFERENCES

1. Shepherd, J.H., Knuppel, R.A.: Role of prostaglandins in ripening the cervix and inducing labor. *Clin. in Perinatol.* 8:49, 1981.
2. Forman, A., Ulmsten, U., Banyai, J., Wingerup, L., and Uldbjerg, N.: Evidence for a local effect of intracervical PGE₂-gel. *Am. J. Obstet. Gynecol.*, in press.
3. Ekman, G., Forman, A., Maršál, K. and Ulmsten, U.: Intravaginal versus intracervical application of prostaglandin E₂ in viscous gel for cervical priming and term labor induction in patients with an unfavorable cervical state. Submitted to *Am. J. Obstet. Gynecol.*
4. Calder, A.A.: The management of the unripe cervix. In: *Human parturition*, first edition. Edited by M. Keirse, A. Anderson, J. Benebroek-Gravenhorst. Leiden University Press p. 201, 1979.
5. Naftolin, F., and Stubblefield, P.-H. G.: *Dilatation of the uterine cervix*. Ed. 1 Ravenpress, New York, 1980.
6. Kleissel, H.P., van der Rest, M., Naftolin, F., Glorieux, F.K., de Leon, A.: Collagen changes in the human uterine cervix at parturition. *Am. J. Obstet Gynecol.* 130:748, 1978.
7. Ito, A., Kitamura, K., Mori, Y., Tirakava, S.: The change in solubility of type I collagen in human uterine cervix in pregnancy at term. *Biochem. Med.* 21:262, 1979.
8. Uldbjerg, N., Malmström, A., Ekman, G., Sheehan, J., Ulmsten, U., Wingerup, L.: Dermatan sulphate proteoglycan from human uterine cervix: Isolation and characterization. Submitted for publication.
9. Scott, J.E., Orford, C.R.: Dermatan sulphate - rich proteoglycan associates with rat tail - tendon collagen at the d-band in the gap region. *Biochem. J.* 197:213, 1981.
10. Kitamura, K., Ito, A., Mori, Y., and Hirakawa, S.: Glycosaminoglycans of human uterine cervix: Heparan sulfate increase with reference to cervical ripening. *Biochem. Med.* 23:159, 1980.
11. Wingerup, L., Andersson, K.-E., and Ulmsten, U.: Ripening of the cervix and induction of labor in patients at term by single intracervical application of prostaglandin E₂ in viscous gel. *Acta Obstet. Gynecol. Scand. Suppl.* 84:11, 1979.
12. Carlson, Don M.: Structures and immunochemical properties of oligosaccharides isolated from pig submaxillary mucines. *J. Biolog. Chem.* 243:616, 1968.
13. Antonopoulos, C.A., Gardell, S., Szirmai, J.A., De Tyssonsk, E.R.: Determination of glycosaminoglycans (mucopolysaccharides) from tissues on the microgram-scale. *Biochem. Biophys. Acta* 83:1, 1964.

14. Stegeman, H., Stalder, K.: Determination of hydroxyproline. *Clin. Chem. Acta* 18:267, 1967.
15. Kitamura, K., Ito, A., Mori, Y.: The existing forms of collagenase in the human uterine cervix. *J. Biochem.* 87:753, 1980.
16. Masui, Y., Takemoto, T., Sakakibara, S., Hori, H., Nagai, Y.: Synthetic substrates for vertebrate collagenase. *Biochem. Med.* 17:215, 1977.
17. Ohlsson, K., Olsson, A.-S.: Immunoreactive granulocyte elastase in human serum. *Hoppe-Seyler's Z. Physiol. Chem.* 359:1531, 1978.
18. Rorie, D.K., Newton, M.: Histologic and chemical studies of the smooth muscle in the human cervix and uterus. *Am. J. Obstet. Gynecol* 99:466, 1967.
19. Richard A. Berg: *Methods in enzymology*. Edited by Leon W. Cunningham and Dixie W. Frederiksen 1982. 82:372, 1982.
20. Kitamura, K., Ito, A., Mori, Y., Hirakava, S.: Changes in the human uterine cervical collagenase with special reference to cervical ripening. *Biochem. Med.* 22:332, 1979.
21. Junqueira, L.C.U., Zugaib, M., Montez, G.S., Toledo, O.M.S., Krisztán, R.M., Shigihara, K.M.: Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilatation. *Am. J. Obstet. Gynecol.* 138:273, 1980.
22. Leppert, P.C., Keller, S., Cerreta, J., Mandl, I.: Conclusive evidence for the presence of elastin in human and monkey cervix. *Am. J. Obstet. Gynecol.* 142:179, 1982.
23. Andersson, K.-E., Forman, A., and Ulmsten, U.: Pharmacology of labor. *Clin. Obstet. Gynecol.* Ed. U. Ulmsten & K. Ueland, March 1983, Harper & Row.

INCREASED COLLAGENOLYTIC ACTIVITY POST PARTUM
IN CERVICAL CONNECTIVE TISSUE FROM WOMEN
TREATED WITH PROSTAGLANDIN E₂

Gunvor Ekman, Niels Uldbjerg,
Anders Malmström, Ulf Ulmsten,

Department of Obstetrics and Gynecology, Malmö General Hospital, University of Lund, Department of Obstetrics and Gynecology, University of Århus, Denmark and Department of Physiological Chemistry, University of Lund, Sweden

ABSTRACT

Cervical biopsies obtained immediately post partum from 7 women induced with intracervical application of 0.5 mg prostaglandin E₂ in viscous gel, were compared with similar biopsies from 11 spontaneously delivered women. A DNP-peptide hydrolytic activity (collagenase) was significantly increased in cervical tissue from the PGE₂ induced patients compared to controls. In patients with prompt clinical response, the increase was nearly twofold. No differences were found in the concentrations of water, sulphated glycosaminoglycans, hyaluronic acid, hydroxyproline or leucocyte elastase. Thus PGE₂ induced cervical priming seems to be associated with an increased collagenolytic activity.

Induction of labor with conventional methods (intravenous infusion of oxytocin and/or amniotomy) in term patients with an unripe cervix is associated with increased risk for foetal distress and high frequency of instrumental deliveries (1). Therefore, there is a need for other methods for management of these patients. Recently, local application of prostaglandin E_2 (PGE_2) in viscous gel has been found an effective treatment for cervical priming and induction of labor in this category of patients (2,3).

The human uterine cervix is dominated by a dense fibrous connective tissue with up to 15 % smooth muscle. The biophysical properties of the organ is mainly determined by the collagen content. Spontaneous cervical ripening during pregnancy and parturition is associated with a gradual fall in the concentrations of collagen and glycosaminoglycans, and a corresponding increase in collagenolytic activity (DNP-peptide hydrolytic activity and leucocyte elastase) (4). The aim of the present investigation was to study these parameters in cervical biopsies obtained from women delivered spontaneously or after PGE_2 treatment, in order to elucidate whether changes herein might explain the cervical softening induced by PGE_2 -treatment.

MATERIAL AND METHOD

Eighteen term pregnant women, 7 treated with PGE_2 -gel and 11 spontaneously delivered who served as controls, participated in the study. All were informed about the aims of the investigation and gave their consent.

The seven women receiving 0.5 mg PGE_2 in gel intracervically had a mean age of 27 years (range 23 - 31), a mean parity of 0.4 (range 0 - 1), a mean gestational age of 40 weeks (range 39 - 43), and a mean cervical score of 4.0 (range 1 - 5). In all there were obstetric indications for induction of labor. Moreover, all patients had head presentations and intact membranes.

For practical reasons the patients were admitted for induction in the morning. After assessment of cervical state according to a modified Bishop score (unfavorable cervical state ≤ 5 points), 0.5 mg PGE_2 in gel was prepared as described elsewhere (2) and applied intracervically using a 12 cm long, 3 mm thick nylon catheter with a hole on one side 0.5 cm from the tip. Starting just beneath the internal os, the gel was gently infused during slow withdrawal of the catheter. Immediately after completed gel application, the catheter was removed. Cardiotocographic registration was performed from 30 minutes before application to at least one hour after gel application.

Cervical state was assessed 5 and 24 hours after gel application, and when favorable cervical state was achiev-

ed, intravenous oxytocin was instituted in patients not in established labor.

The control group consisted of 11 women with a mean age of 27 years (range 18 - 36), a mean parity of 0.1 (range 0 - 1), and a mean gestational age of 40 weeks (range 37 - 42).

At the time of admission, the controls had established labor with regular uterine contractions since 2 to 4 hours and cervix dilated to about 2 cm.

To be able to compare the course of delivery, the time for cervical dilatation from 2 to 10 cm was registered in both groups. In neither group was amniotomia performed until the cervix was dilated > 4 cm and labor well established. Immediately after delivery, cervical biopsies weighing 50 - 100 mg were obtained by using tweezers and scissors. The specimens were frozen in liquid nitrogen within 3 minutes and stored at -60°C .

The biopsies were analyzed for DNP-peptide hydrolytic activity and concentrations of leucocyte elastase, hydroxyproline, sulphated glycosaminoglycans, hyaluronic acid, and water.

Analytical techniques

Water and glycosaminoglycans

The water content of the biopsies was defined as the

difference between the wet weight and the dry weight. The latter was determined after heating the sample at 110°C in vacuum over P_2O_5 for at least five hours, after which a constant weight was obtained. The dry sample was delipidated with acetone and digested with papain. The digest was applied at a column with 1 ml DEAE cellulose in acetate form. Hyaluronic acid was eluted with 1.1 M pyridine acetate pH 5 and sulphated glycosaminoglycans with 3.0 M pyridine acetate pH 5, and lyophilized. The fraction containing hyaluronic acid was subjected to alkaline degradation according to Carlsson (5) and applied to a column containing 0.5 ml DEAE cellulose. Oligosaccharides from mucin glycopeptides were eluted with 0.4 M pyridine acetate and purified hyaluronic acid with 1.2 M pyridine acetate. Sulphated glycosaminoglycans and hyaluronic acid were hydrolyzed in 6 M HCl, 100°C for eight hours, lyophilized and quantitated as hexosamine (6).

Collagen concentration and extractability: The biopsies were cut into pieces smaller than 2 mm and delipidated with acetone. They were initially extracted three times with 1 ml 0.5 M acetic acid and then three times with 1 ml 0.5 M acetic acid containing pepsin (1 mg/ml) (7). Extractions (23 hours) and centrifugations (one hour at 20,000 x g) between these were performed at 4°C. The material was hydrolyzed in 2 ml 6 M HCl at 100°C for 17 hours, lyophi-

lized and quantitated as hydroxyproline according to Stegeman (8).

Collagenolytic activity: Extractions were performed essentially as described by Kitamura (9). The biopsy was crushed by 10 tons of pressure during cooling with liquid nitrogen and then homogenized in 13 volumes of 0.25 % Triton-X100, 50 mM Tris pH 7.3 containing 100 mM CaCl_2 in a TRI-R Tissue homogenizer, S61 for four minutes at 3,000 rpm. The homogenate was centrifuged at 15,000 x g for 20 minutes at 2°C, whereafter the supernatant was diluted with extraction buffer. The hydrolytic activity against DNP-peptide was then assayed as described by Masui et al. (10). The incubation time was 2 hours and the extraction buffer was used as blank.

Leucocyte elastase was determined in the same extractions with a RIA-method (11).

Chemical reagents

Papain (twice crystallized, 16 - 40 BAAE units/mg) was purchased from Sigma Chemical Co., U.S.A., DEAE cellulose ion exchanger (DE-52) from Whatman Ltd., England, and 2.4 dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg (DNP-peptide) from the Protein Research Foundation, Japan. All other chemicals were of analytical grade.

RESULTS

The results are summarized in Table I. A favorable cervical state was registered in 6 out of 7 PGE₂-treated patients 5 hours after application of the PGE₂-gel (cervix score changed from 3.5→7.1). Four of these six patients had regular uterine contractions at that time, whereas the other two were stimulated with oxytocin. All six were delivered vaginally within 12 hours after the gel application. The woman with unfavorable cervix after 5 hours started labor without further stimulation 14 hours after the gel application.

All 11 women in the control group were delivered vaginally without complications within 15 hours. The mean cervical dilatation time was 5.6 hours in the PGE₂-treated group compared to 7.4 hours for the controls. This difference is statistically significant $p < 0.001$ (t-test).

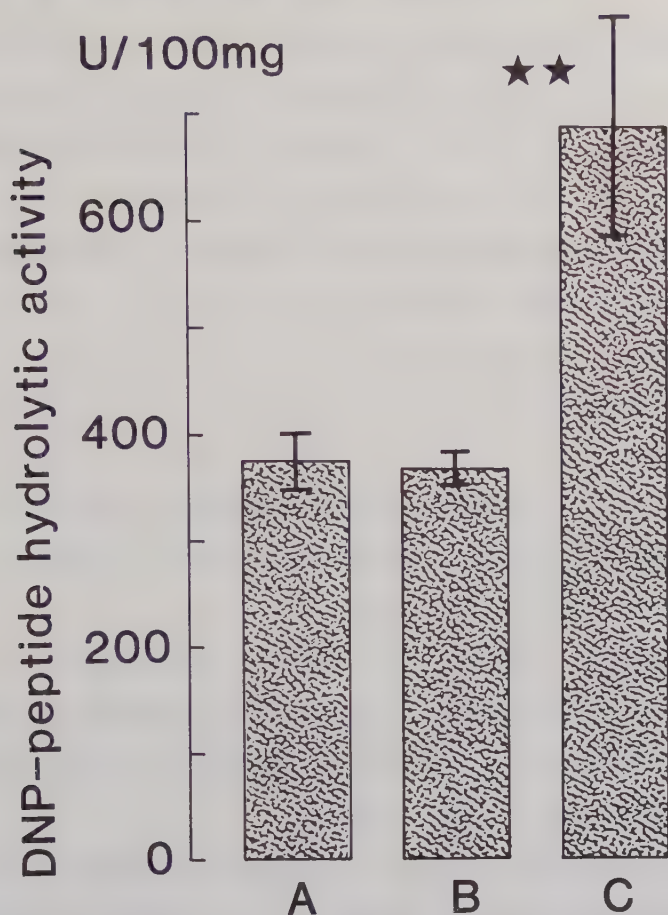
In the cervical biopsies obtained from the PGE₂-treated patients, the DNP-peptide hydrolytic activity (collagenase) was 550 U/100 mg versus 372 U/100 mg in the controls, $p < 0.05$ (t-test Table I).

In the four patients with prompt response to the treatment, i.e., favorable cervical state combined with established labor after 5 hours, the DNP-peptide hydrolytic activity

Table 1

Composition of the uterine cervix immediately post partum in women delivered spontaneously (controls) and in women induced with PGE₂-gel (PGE₂-induced). All values are given as mean $\pm$ s.e.m. *, different from control ($p < 0.05$, t-test).

	Controls	PGE ₂ -induced
Hydroxyproline		
Total ($\mu\text{g}/\text{mg}$ wet weight)	$6.7^{\pm}0.6$	$6.2^{\pm}1.3$
Extractability in HAc (%)	$0.9^{\pm}0.1$	$0.9^{\pm}0.01$
Extractability in "pepsin" (%)	$88^{\pm}1.4$	$87^{\pm}1.2$
Water (%)		
	$87^{\pm}0.5$	$84^{\pm}1.3$
Sulphated glycosaminoglycans		
(μg hexosamine/ mg wet weight)	$0.16^{\pm}0.03$	$0.11^{\pm}0.02$
Hyaluronic acid		
(μg hexosamine/ mg wet weight)	$0.06^{\pm}0.02$	$0.08^{\pm}0.01$
DNP-peptide hydrolytic activity		
(U/100 mg)	$372^{\pm}27$	$550^{\pm}86^*$
Leucocyte elastase (ng/mg wet weight)		
	$6.8^{\pm}0.4$	$6.5^{\pm}0.2$



A : Control

B : Moderate clinical response

C : Prompt clinical response

Fig. 1 The DNP-peptide hydrolytic activity in cervical tissue from PGE₂-induced (prompt and moderate responders) and spontaneously delivered patients (controls). The specimens were taken immediately after delivery. Each bar indicates S.E.M.**, Different from control, $p < 0.01$ (t-test). See also text.

was highly significantly increased (688 U/100 mg versus 372 U/100 mg $p < 0.01$, t -test. Fig. 1).

No significant difference was found between the two groups concerning the concentrations of hydroxyproline (collagen), sulphated glycosaminoglycans, hyaluronic acid, water, and leucocyte elastase (Table I).

DISCUSSION

Normal cervical ripening during pregnancy and parturition is associated with pronounced biochemical changes within the cervical connective tissue (4).

As in previous studies, the present investigation showed that intracervical application of 0.5 mg PGE₂ in gel can within a short time prime the unfavorable cervix prior to term labor induction (12,13).

One important factor associated with the pharmacologically induced priming is most likely the increased collagenolytic activity suggesting cleavage of collagen fibrils. This statement is further underlined by the fact that in patients with an excellent clinical effect of the PGE₂ gel treatment, the DNP-peptide hydrolytic activity was significantly increased compared to that found in those with moderate clinical response.

Despite a high collagenolytic activity, the collagen concentration was not significantly lower in the PGE₂-gel treated patients compared to the controls (6.2 ± 1.3 $\mu\text{g}/\text{mg}$ wet weight versus 6.7 ± 0.6 $\mu\text{g}/\text{mg}$ wet weight). This might

be explained by the fact that the degraded collagen for some time is retained within the cervical tissue as suggested by Kleissl et al. (14).

The experimental protocol used in this study was not optimal since the control group and the PGE_2 -treated group were not equivalent. The control patients should have had more favorable cervixes indicated by the spontaneous onset of labor. Despite this fact, the PGE_2 -treated group displayed a highly significant increase in the collagenolytic activity compared to those of the women in the control group.

Thus the high DNP-peptide hydrolytic activity (collagenase) seems to be well correlated with the marked clinical response to PGE_2 -treatment (Fig. 1). It is therefore reasonable to believe that a PGE_2 -induced collagenase activity is important for the cervical ripening process.

REFERENCES

1. Calder, A.A.: The management of the unripe cervix. Human parturition, First edition. Ed. by M. Keirse, A. Anderson, J. Bennebroek - Gravenhorst. Leyden University Press p. 201, 1979.
2. Ulmsten, U., Wingerup, L.: Cervical ripening induced by prostaglandin E_2 in viscous gel. Acta Obstet Gynecol Scand Suppl 84, 1979.
3. Shepherd, J.H., Knuppel, R.A.: Role of prostaglandins in ripening in the cervix and inducing labor. Clin in Perinatol 8:49, 1981.
4. Uldbjerg, N., Ekman, G., Malmström, A., Olsson, K., and Ulmsten, U.: Ripening of the human cervix related to changes in collagen glycosaminoglycans and collagenolytic activity. Submitted for publication.
5. Carlson, Don M.: Structures and immunochemical properties of oligosaccharides isolated from pig submaxillary mucins. J Biolog Chem 243:616, 1968.
6. Antonopoulos, C.A., Gardell, S., Szirmai, J.A., de Tyssensk, E.R.: Determination of glycosaminoglycans (mucopolysaccharides) from tissues on the microgram scale. Biochim Biophys Acta 83:1, 1964.
7. Ito, A., Kitamura, K., Mori, Y., Hirakawa, S.: The change in solubility of type I collagen in human uterine cervix in pregnancy at term. Biochem Med 21:262, 1979.
8. Stegeman, H., Stalder, K.: Determination of hydroxyproline. Clin Chim Acta 18:267, 1967.
9. Kitamura, K., Ito, A., Mori, Y.: The existing forms of collagenase in the human uterine cervix. J Biochem 87:753, 1980.
10. Masui, Y., Takemoto, T., Sakakibara, S., Hori, H., Nagai, Y.: Synthetic substrates for vertebrate collagenase. Biochem Med 17:215, 1977.
11. Ohlsson, K., Olsson, A.-S.: Immunoreactive granulocyte elastase in human serum. Hoppe Seyler's Z Physiol Chem 359:1531, 1978.
12. Ulmsten, U., Wingerup, L.: Clinical experiences with a new gel for intracervical application of prostaglandin E_2 before therapeutic abortion or induction of term labour. Prostaglandins 20:533, 1980.
13. Ulmsten, U., Wingerup, L., Belfrage, P., Ekman, G., and Wikqvist, N.: Intracervical application of prostaglandin gel for induction of term labor. Obstet Gynecol 59:336, 1982.
14. Kleissl, H.P., van der Rest, M., Naftolin, F., Glorieux, F.H., De Leon, A.: Collagen changes in the human uterine cervix at parturition. Am J Obstet Gynecol 130:748, 1978.

